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St. Geme, III et al.

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(54) **HAEMOPHILUS ADHERENCE AND PENETRATION PROTEINS**

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(65) **Prior Publication Data**

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Related U.S. Application Data

(62) Division of application No. 09/839,996, filed on Apr. 20, 2001, now Pat. No. 6,642,371, which is a division of application No. 08/296,791, filed on Aug. 25, 1994, now Pat. No. 6,245,337.

(51) **Int. Cl.**⁷ **C12P 21/06**

(52) **U.S. Cl.** **435/69.1**; 435/69.3; 435/70.1; 435/70.3; 435/71.1

(58) **Field of Search** 435/69.1, 69.3, 435/70.1, 70.3, 71.1, 320.1, 325, 243, 252.3, 254.2, 348, 367, 372; 536/23.7

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(57) **ABSTRACT**

Haemophilus adhesion and penetration proteins, nucleic acids, vaccines and monoclonal antibodies are provided.

13 Claims, 19 Drawing Sheets

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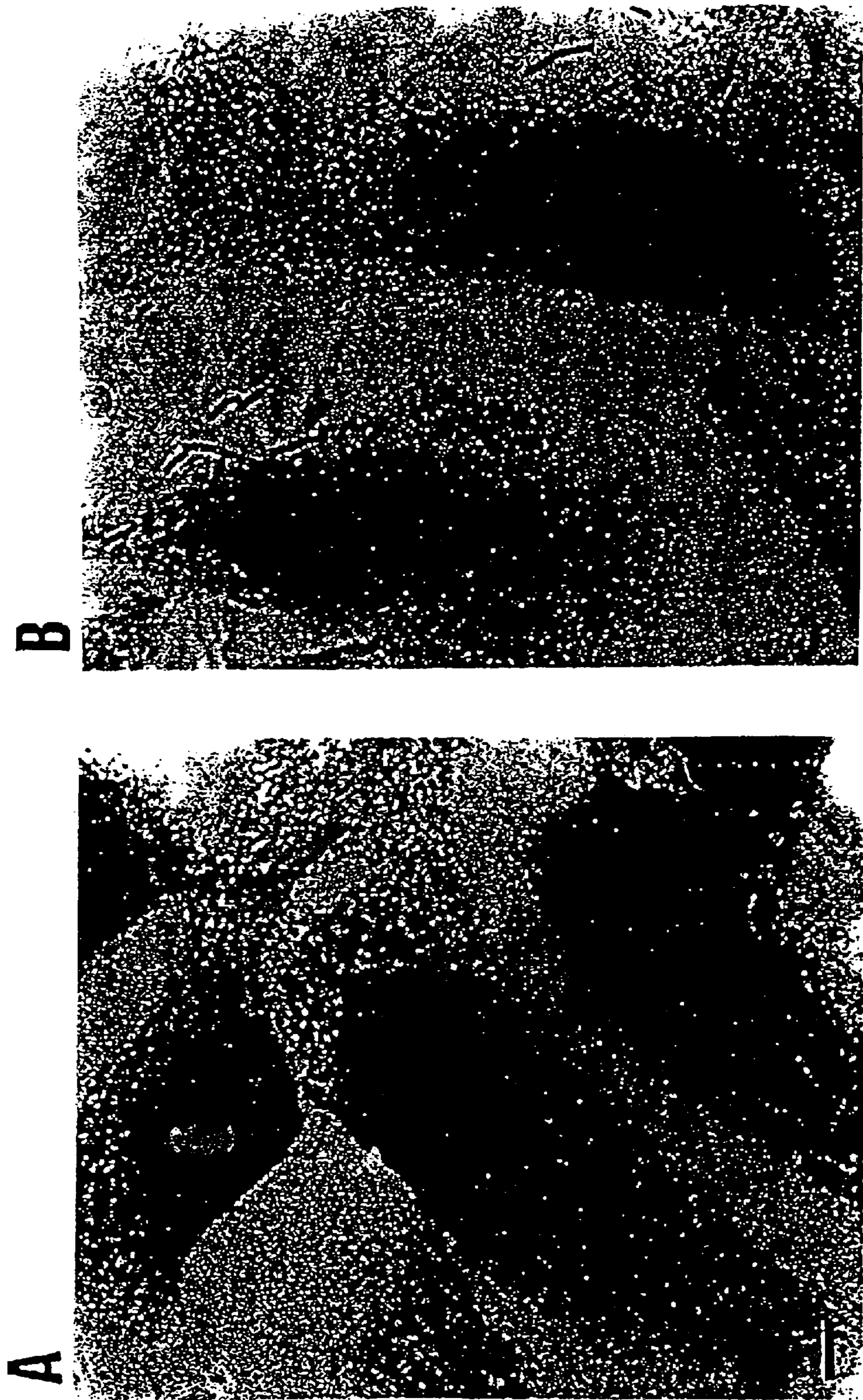
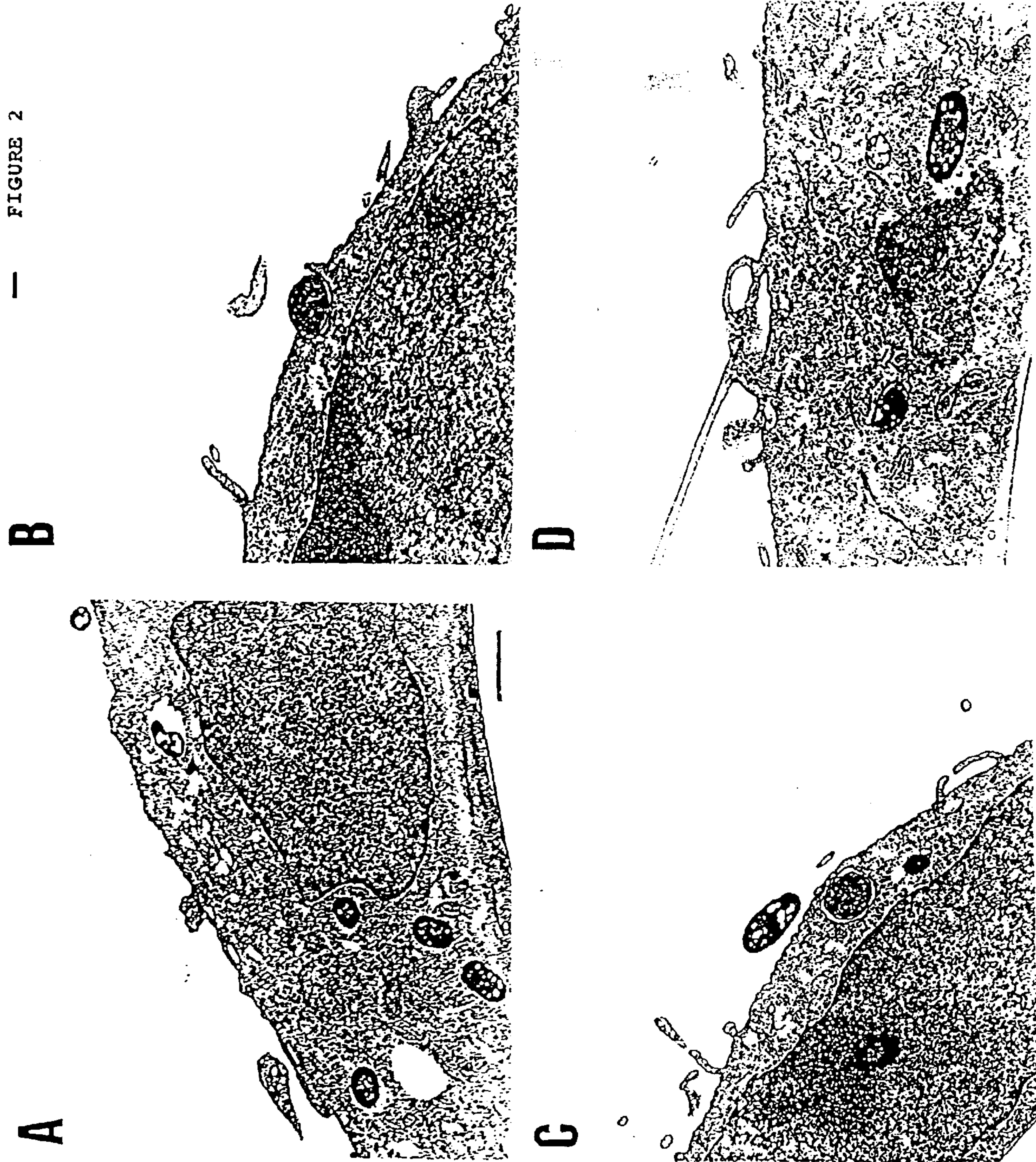


FIGURE 1

FIGURE 2



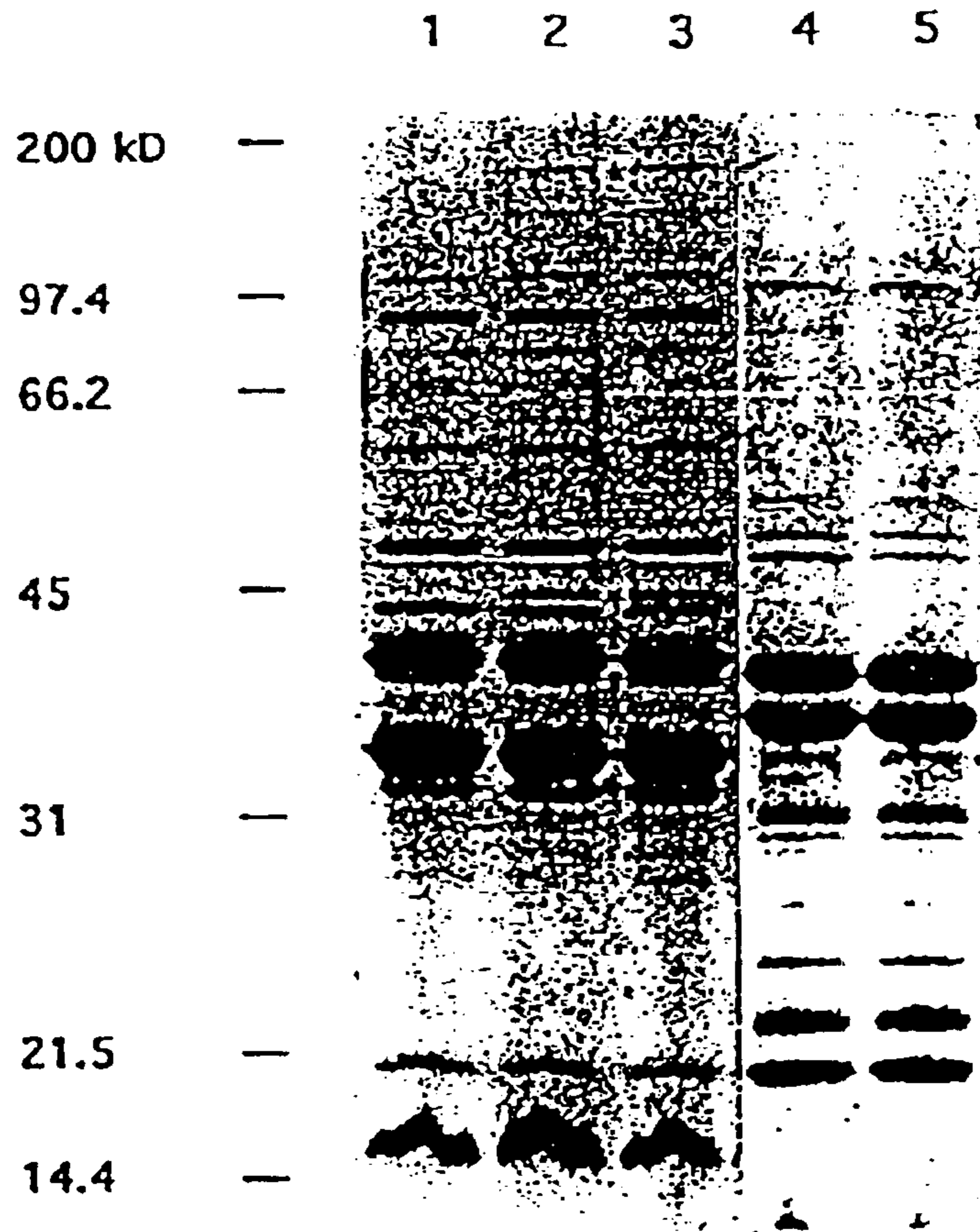


FIGURE 3

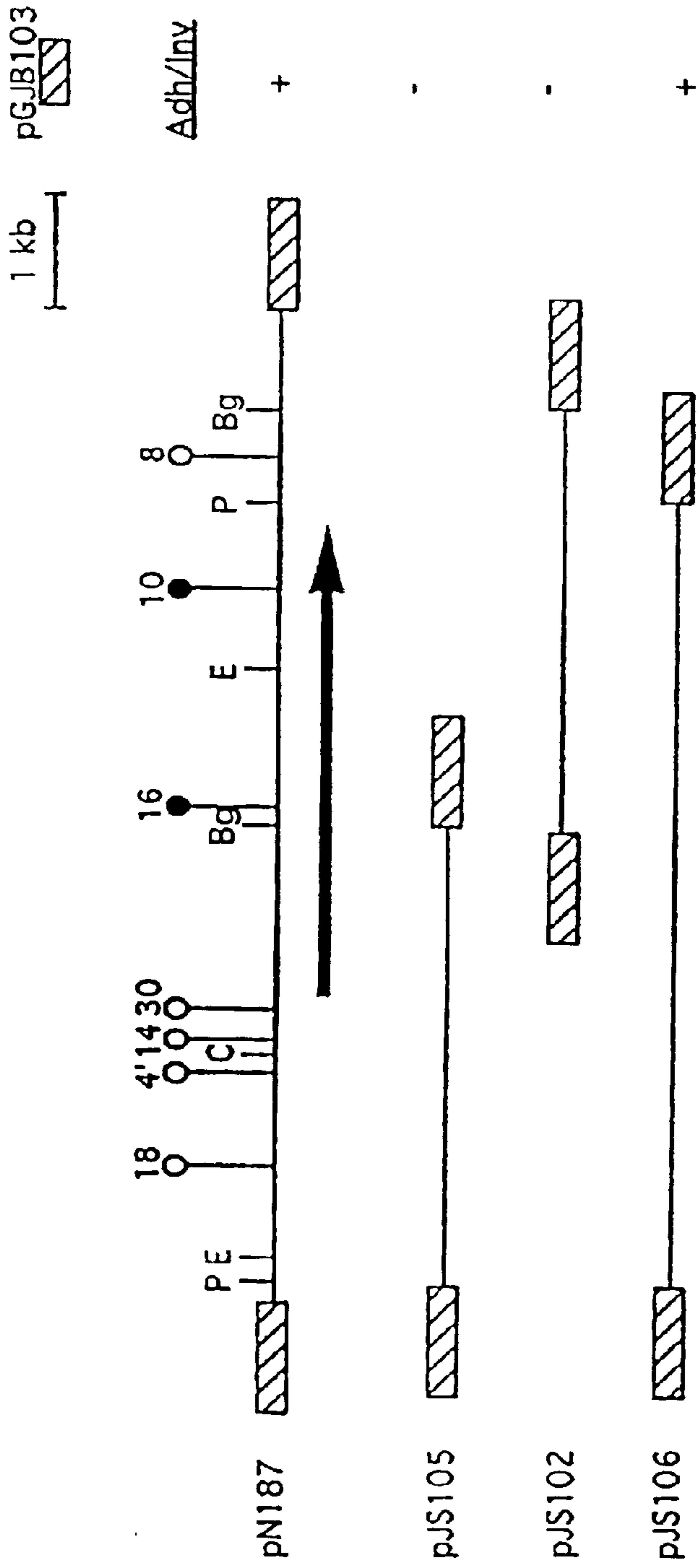


FIGURE 4

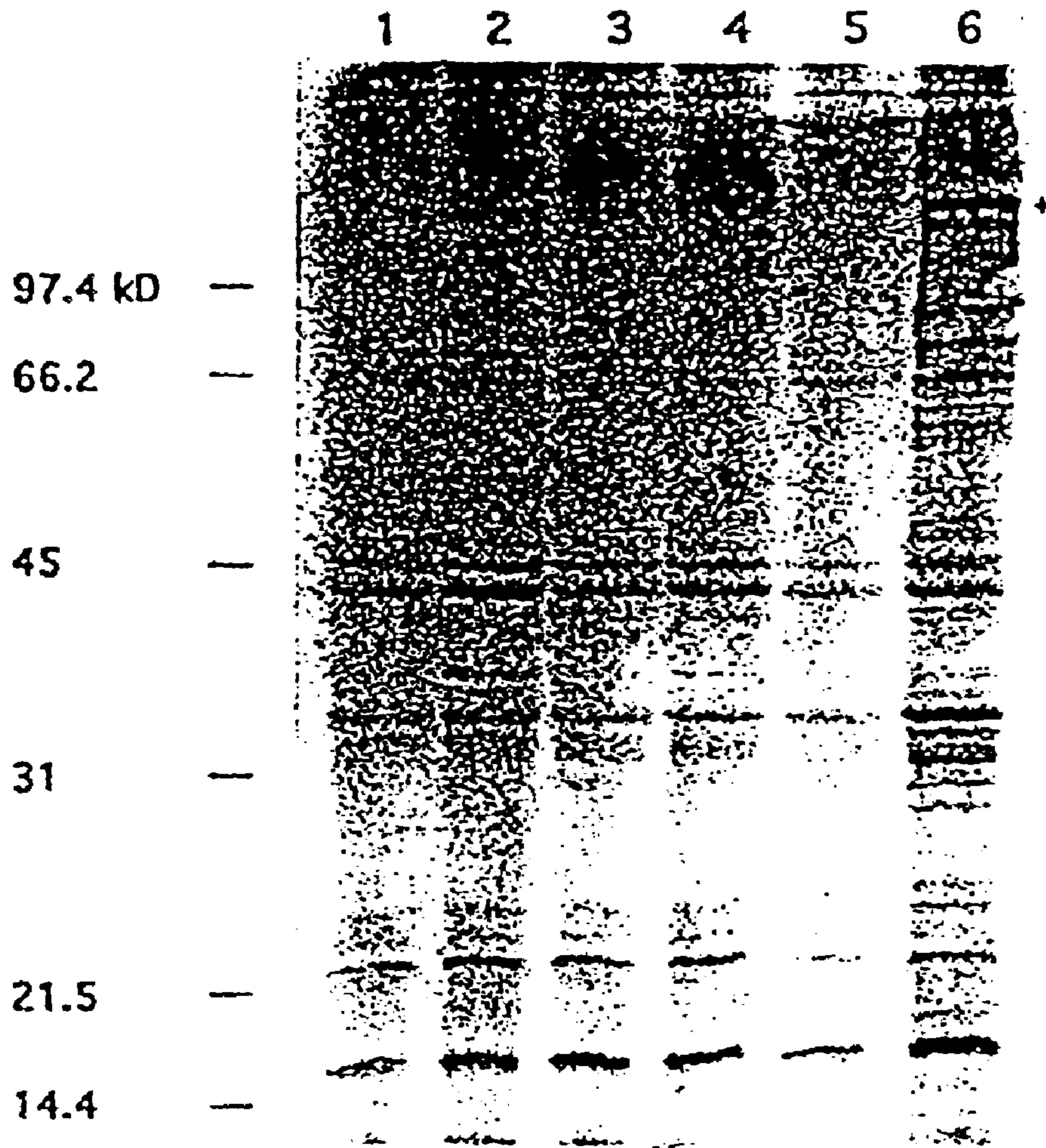


FIGURE 5

10 30 50 70 90
 AATAGTCGTTTAACTAGTATTTTTTAAATACGAAAAATTACTTAATTAATAAACATTATGAAAAAACTGTATTTTCGTCTTAATTTT
 -35 -10 M K K T V F R L N F
 110 130 150 170
 AACCGCTTGCATTTTATTAGGGATAGTATCGCAAGCGTGGGCTGGTCACACTTATTTTGGGATTGATTACCAATATTATCGTGATTTT
 T A C I S L G I V S Q A W A G H T Y F G I D Y Q Y Y R D F
 190 210 230 250 270
 CGAGAATAAAGGGAAGTTCACAGTTGGGGCTCAAATATTAAGTTTATAACAAACAAGGGCAATTAGTTGGCACATCAATGACAAAA
 E N K G K F T V G A Q N I K V Y N K Q G Q L V G T S M T K
 290 310 330 350
 CCCGATGATTGATTTTTCTGTAGTGTACGTAACGGCGTGGCAGCCTTGGTTGAAAATCAATATATTGTGAGCGTGGCACATAACGTA
 P M I D F S V V S R N G V A A L V E N Q Y I V S V A H N V
 370 390 410 430 450
 ATATACAGATGTTGATTTTGGTGCAGAGGAAACAACCCCGATCAACATCGTTTTACTTATAAGATTGTAACGAAATAACTACAAA
 Y T D V D F G A E G N N P D Q H R F T Y K I V K R N N Y K
 470 490 510 530
 AAGATAATTTACATCCTTATGAGGACGATTACCATAATCCACGATTACATAAATTCGTTACAGAAGCGGCTCCAATTGATATGACTTCG
 D N L H P Y E D D Y H N P R L H K F V T E A A P I D M T S
 550 570 590 610 630
 ATATGAATGGCAGTACTTATTCAGATAGAACAATAATCCAGAACGTGTTTCGTATCGGCTCTGGACGGCAGTTTTGGCGAAATGATCAA
 M N G S T Y S D R T K Y P E R V R I G S G R Q F W R N D Q
 650 670 690 710
 ACAAGGGCGACCAAGTTGCCGGTGCATATCATTATCTGACAGCTGGCAATACACACAATCAGCGTGGAGCAGGTAATGGATATTCGTAT
 K G D Q V A G A Y H Y L T A G N T H N Q R G A G N G Y S Y
 730 750 770 790 810
 TGGGAGGCGATGTTTCGTAAAGCGGGAGAATATGGTCCATTACCGATTGCAGGCTCAAAGGGGGACAGTGGTTCTCCGATGTTTATTTAT
 G G D V R K A G E Y G P L P I A G S K G D S G S P M F I Y
 830 850 870 890
 ATGCTGAAAAACAAAATGGTTAATTAATGGGATATTACGGGAAGGCAACCCCTTTGAAGGCAAAGAAAATGGGTTTCAATTGGTTTCG
 A E K Q K W L I N G I L R E G N P F E G K E N G F Q L V R
 910 930 950 970 990
 AATCTTATTTTATGAAATTTTCGAAAGAGATTTACATACATCACTTTACACCCGAGCTGGTAATGGAGTGTACACAATTAGTGAAAT
 C S Y F D E I F E R D L H T S L Y T R A G N G V Y T I S G N
 1010 1030 1050 1070
 GATAATGGTCAGGGTCTATAACTCAGAAATCAGGAATACCATCAGAAATTAATAATACGTTAGCAAATATGAGTTTACCTTTGAAAGAG
 D N G Q G S I T Q K S G I P S E I K I T L A N M S L P L K E
 1090 1110 1130 1150 1170
 AAGGATAAAGTTCATAATCCTAGATATGACGGACCTAATATTTATCTCCACGTTTAAACAATGGAGAAACGCTATATTTTATGGATCAA
 K D K V H N P R Y D G P N I Y S P R L N N G E T L Y F M D Q
 1190 1210 1230 1250
 AAACAAGGATCATTAAATCTTCGCATCTGACATTAACCAAGGGGCGGGTGGTCTTTATTTTGGAGGTAATTTTACAGTATCTCCAAATCT
 K Q G S L I F A S D I N Q G A G G L Y F E G N F T V S P N S
 1270 1290 1310 1330 1350
 AACCAAACTTGGCAAGGAGCTGGCATAATGTAAGTGAATAAGTACACCGTTACTTGGAAAGTAAATGGCGTGGAACATGATCGACTTTCT
 N Q T W Q G A G I H V S E N S T V T W K V N G V E H D R L S
 1370 1390 1410 1430
 AAAATTGGTAAAGGAACATTGCACGTTCAAGCCAAAGGGGAAAATAAAGGTTTCGATCAGCGTAGGCGATGGTAAAGTCATTTTGGAGCAG
 K I G K G T L H V Q A K G E N K G S I S V G D G K V I L E Q



FIGURE 6A

1450 1470 1490 1510 1530
 AGGCAGACGATCAAGGCAACAAACAAGCCTTTAGTGAAATTGGCTTGGTTAGCGGCAGAGGGACTGTTCAATTAACGATGATAAACAA
 A D D Q G N K Q A F S E I G L V S G R G T V Q L N D D K Q

 1550 1570 1590 1610
 TTGATACCGATAAATTTTATTTCCGGCTTTTCGTGGTGGTTCGCTTAGATCTTAACGGGCATTTCATTAACCTTTAAACGTATCCAAAATACG
 D T D K F Y F G F R G G R L D L N G H S L T F K R I Q N T

 1630 1650 1670 1690 1710
 TACGAGGGGGCAATGATTGTGAACCATAATACTCAAGCCGCTAATGTCCTACTTACTGGGAACGAAAGCATTGTTTETACCTAATGGA
 E G A M I V N H N T T Q A A N V T I T G N E S I V L P N G

 1730 1750 1770 1790
 TATAATATTAATAAACTTGATTACAGAAAAGAAATGCCTACAACGGTTGGTTTGGCGAAACAGATAAAAAATAACACAATGGGCGATTA
 I N I N K L D Y R K E I A Y N G W F G E T D K N K H N G R L

 1810 1830 1850 1870 1890
 TACCTTATTTATAAACCAACCACAGAAGATCGTACTTTGCTACTTTTCAGGTGGTACAAATTTAAAAGGCGATATTACCCAAACAAAAGGT
 V L I Y K P T T E D R T L L L S G G T N L K G D I T Q T K G

 1910 1930 1950 1970
 TAACTATTTTTTCAGCGGTAGACCGACACCGCACGCCTACAATCATTTAAATAAACGTTGGTCAGAAATGGAAGGTATACCACAAGGCGAA
 K L F F S G R P T P H A Y N H L N K R W S E M E G I P Q G E

 1990 2010 2030 2050 2070
 ATTGTGTGGGATCACGATTGGATCAACCGTACATTTAAAGCTGAAAACCTTCCAAATTAAGGCGGAAGTGCGGTGGTTTCTCGCAATGTT
 I V W D H D W I N R T F K A E N F Q I K G G S A V V S R N V

 2090 2110 2130 2150
 TCTTCAATTGAGGGAAATTGGACAGTCAGCAATAATGCAAATGCCACATTTGGTGTGTGCCAAATCAACAAAATACCATTTGCACGCGT
 S S I E G N W T V S N N A N A T F G V V P N Q Q N T I C T R

 2170 2190 2210 2230 2250
 TCAGATTGGACAGGATTAACGACTTGTCAAAAAGTGGATTTAACCGATACAAAAGTTATTAATTTCTATACCAAAAACACAAATCAATGGC
 S D W T G L T T C Q K V D L T D T K V I N S I P K T Q I N G

 2270 2290 2310 2330
 TCTATTAATTTAACTGATAATGCAACGGCGAATGTTAAAGGTTTAGCAAAAACCTTAATGGCAATGTCCTTTAACAANTCACAGCCAATTT
 S I N L T D N A T A N V K G L A K L N G N V T L T N H S Q F

 2350 2370 2390 2410 2430
 ACATTAAGCAACAATGCCACCCAAATAGGCAATATTCGACTTTCCGACAATCAACTGCAACGGTGGATAATGCAAACTTGAACGGTAAT
 T L S N N A T Q I G N I R L S D N S T A T V D N A N L N G N

 2450 2470 2490 2510
 GTGCATTTAACGGATTGAGCTCAATTTTCTTTAAAAAACAGCCATTTTTTCGCACCAAATTCAGGGAGACAAAGGCACAACAGTGACGTTG
 V H L T D S A Q F S L K N S H F S H Q I Q G D K G T T V T L

 2530 2550 2570 2590 2610
 GAAAATGCGACTTGGACAATGCCTAGCGATACTACATTGCAGAATTTAACGCTAAATAACAGTACGATCACGTTAAATTCAGCTTATTCA
 E N A T W T M P S D T T L Q N L T L N N S T I T L N S A Y S

 2630 2650 2670 2690
 GCTAGCTCAAACAATACGCCACGTGCGCGTTTATTAGAGACGGAAACAACGCCAACATCGGCAGAACATCGTTTCAACACATTGACAGTA
 A S S N N T P R R R S L E T E T T P T S A E H R F N T L T V

 2710 2730 2750 2770 2790
 AATGGTAAATTGAGTGGGCAAGGCACATTCCAATTTACTTCATCTTTATTTGGCTATAAAAGCGATAAATTAATTAATCAATGACGCT
 N G K L S G Q G T F Q F T S S L F G Y K S D K L K L S N D A

 2810 2830 2850 2870
 GAGGGCGATTACATATTATCTGTTTCGCAACACAGGCAAAGAACCCTTGGCAATTAACCTTTGGTTGAAAGCAAAGATAATCAA
 E G D Y I L S V R N T G K E P E T L E Q L T L V E S K D N Q

— **FIGURE 6B** —

2890 2910 2930 2950 2970
 CGTTATCAGATAAGCTCAAATTTACTTTAGAAAATGACCACGTTGATGCAGGTGCATTACGTTATAAATTAGTGAAGAATGATGGCGAA
 L S D K L K F T L E N D H V D A G A L R Y K L V K N D G E

 2990 3010 3030 3050
 TCCGCTTGATAACCCAATAAAAGAGCAGGAATTGCACAATGATTTAGTAAGAGCAGAGCAAGCAGAACGAACATTAGAAGCCAAACAA
 R L H N P I K E Q E L H N D L V R A E Q A E R T L E A K Q

 3070 3090 3110 3130 3150
 TTGAACCGACTGCTAAAACACAAACAGGTGAGCCAAAAGTGCGGTCAAGAAGAGCAGCGAGAGCAGCGTTTCTGATACCTGCCTGAT
 E P T A K T Q T G E P K V R S R R A A R A A F P D T L P D

 3170 3190 3210 3230
 CAAAGCCTGTTAAACGCATTAGAAGCCAAACAAGCTGAACTGACTGCTGAAACACAAAAAGTAAGGCAAAAACAAAAAAGTGCGGTCA
 S L L N A L E A K Q A E L T A E T Q K S K A K T K K V R S

 3250 3270 3290 3310 3330
 AAAAGAGCAGTGTTCCTGATCCCCTGCTTATCAAAGCCTGTTGCGATTAGAAGCCGCACTTGAGGTTATTGATGCCCCACAGCAATCG
 K R A V F S D P L L D Q S L F A L E A A L E V I D A P Q Q S

 3350 3370 3390 3410
 GAAAAAGATCGTCTAGCTCAAGAAGAAGCGGAAAAACAACGCAACAAAAAGACTTGATCAGCCGTTATTCAAATAGTGCCTTATCAGAA
 E K D R L A Q E E A E K Q R K Q K D L I S R Y S N S A L S E

 3430 3450 3470 3490 3510
 TTATCTGCAACAGTAAATAGTATGCTTTCTGTTCAAGATGAATTAGATCGTCTTTTTGTAGATCAAGCACAATCTGCCGTGTGGACAAAT
 L S A T V N S M L S V Q D E L D R L F V D Q A Q S A V W T N

 3530 3550 3570 3590
 ATCGCACAGGATAAAAAGACGCTATGATTCTGATGCGTTCGTTTCCGTGCTTATCAGCAGCAGAAAAACGAACTTACGTCAAATTTGGGGTGCAAAAA
 I A Q D K R R Y D S D A F R A Y Q Q Q K T N L R Q I G V Q K

 3610 3630 3650 3670 3690
 GCCTTAGCTAATGGACGAATTGGGGCAGTTTTCTCGCATAGCCGTTTCAAGATAATACCTTTGATGAACAGGTTAAAAATCACGCGAATTA
 A L A N G R I G A V F S H S R S D N T F D E Q V K N H A T L

 3710 3730 3750 3770
 ACGATGATGTCGGGTTTTGCCCAATATCAATGGGGCGATTTACAATTTGGTGTAACGTGGGAACGGGAATCAGTGCGAGTAAATGGCT
 T M M S G F A Q Y Q W G D L Q F G V N V G T G I S A S K M A

 3790 3810 3830 3850 3870
 GAAGAACAAGCCGAAAAATTCATCGAAAAGCGATAAATTATGGCGTGAATGCAAGTTATCAGTTCGTTTAGGGCAATTGGGCATTGAG
 E E Q S R K I H R K A I N Y G V N A S Y Q F R L G Q L G I Q

 3890 3910 3930 3950
 CCTTATTTTGGAGTTAATCGCTATTTTATTGAACGTGAAAATTATCAATCTGAGGAAGTGAGAGTGAAAACGCTAGCCTTGCATTTAAT
 P Y F G V N R Y F I E R E N Y Q S E E V R V K T P S L A F N

 3970 3990 4010 4030 4050
 CGCTATAATGCTGGCATTGAGTTGATTATACATTTACTCCGACAGATAATATCAGCGTTAAGCCTTATTTCTTCGTCAATTATGTTGAT
 R Y N A G I R V D Y T F T P T D N I S V K P Y F F V N Y V D

 4070 4090 4110 4130
 GTTTCAAACGCTAACGTACAAACCACGGTAAATCTCACGGTGTGCAACAACCATTTGGACGTTATTGGCAAAAAGAAGTGGGATTAAG
 V S N A N V Q T T V N L T V L Q Q P F G R Y W Q K E V G L K

 4150 4170 4190 4210 4230
 GCAGAAATTTACATTTCCAAATTTCCGCTTTTATCTCAAATCTCAAGGTTCACTCGGCAACAGCAAAATGTGGGCGTGAAATG
 A E I L H F Q I S A F I S K S Q G S Q L G K Q Q N V G V K L

 4250 4270 4290 4310
 GGCTATCGTTGGTAAAAATCAACATAATTTTATCGTTTATTGATAAACAAGGTGGGTGAGATCAGATCCCACCTTTTATTCCAATAAT
 G Y R W *

— **FIGURE 6C** —

	1				50
Hap	MKKTVERLNF	LTACISLGI	V SQAWAGHTYF	GIDYQYYRDF	AENKGGKFTVG
HK368IGA	MLNKKFKLNF	IALTVAAYALT	PYTEAALVRD	DVDYQIFRDF	AENKGGKFSVG
HK393IGA	MLNKKFKLNF	IALTVAAYALT	PYTEAALVRD	DVDYQIFRDF	AENKGGKFSVG
HK715IGA	MLNKKFKLNF	IALTVAAYALT	PYTEAALVRD	DVDYQIFRDF	AENKGRFSVG
HK61IGA	MLNKKFKLNF	IALTVAAYALT	PYTEAALVRD	DVDYQIFRDF	AENKGGKFSVG
Consensus	M----F-LNF	-----	----A-----	--DYQ--RDF	AENKG-F-VG
	51				100
Hap	AQNIKVYNKQ	GQLVGTSMTK	A.PMIDFSV	SRNG.VAALV	ENQYIVSVAH
HK368IGA	ATNVLVKDKN	NKDLGTALPN	GIPMIDFSV	DVDKRIATLI	NPQYVVGVKH
HK393IG	ATNVEVRDKN	NRPLGNVLPN	GIPMIDFSV	DVDKRIATLV	NPQYVVGVKH
HK715IGA	ATNVEVRDKN	NHSLGNVLPN	GIPMIDFSV	DVDKRIATLI	NPQYVVGVKH
HK61IGA	ATNVEVRDKN	NQSLGSALPN	GIPMIDFSV	DVDKRIATLV	NPQYVVGVKH
Consensus	A-N--V--K-	----G-----	--PMIDFSV	-----A-L-	--QY-V-V-H
	101				150
HapNVGY	TDVDFGAEGN	NPQHR....	..FTYKIVKR	NNY.....
HK368IGA	VSNGVSELHF	GNLNGNMNG	NAKAHROVSS	EENRYFSVEK	NEYPTKLNK
HK393IGA	VSNGVSELHF	GNLNGNMNG	NAKAHROVSS	EENRYYTVEK	NEYPTKLNK
HK715IGA	VSNGVSELHF	GNLNGNMNG	NDKSHROVSS	EENRYFSVEK	NEYPTKLNK
HK61IGA	VSNGVSELHF	GNLNGNMNG	NAKSHROVSS	EENRYYTVEK	NNEPTENVTS
Consensus	-----	-----	N--HR----	----Y--V--	N-----
	151				200
HapKQDNLH	PYEDDYHNPR	LHKEVTEAAP	IDM.TSNMNG	STYSDRTKYP
HK368IGA	TVTTEdq.TQ	KRREDYMPR	LDKEVTEVAP	IEASTASSDA	GTYNQNKYP
HK393IGA	AVTTEdq.AQ	KRREDYMPR	LDKEVTEVAP	IEASTDSSTA	GTYNKDKYP
HK715IGA	AVTTEdq.TQ	KRREDYMPR	LDKEVTEVAP	IEASTASSDA	GTYNQNKYP
HK61IGA	FTTKEEQDAQ	KRREDYMPR	LDKEVTEVAP	IEASTANNNK	GEYNNSDKYP
Consensus	-----	----DY--PR	L-KEVTE-AP	I---T-----	--Y----KYP
	201				250
Hap	ERVRLGSGRQ	F.....WRNDQ	DKGDQVAGAY
HK368IGA	AFVRLGSGSQ	FIIYKKGDNYS	LIL.....N	NH....EVGG	NNLKLVDGAY
HK393IGA	YFVRLGSGTQ	FIIYENGTRYE	LWL.....G	KEGQKSDAGG	YNLKLVDGAY
HK715IGA	AFVRLGSGSQ	FIIYKKGDNYS	LIL.....N	NH....EVGG	NNLKLVDGAY
HK61IGA	AFVRLGSGSQ	FIIYKKGSRVQ	LILTEKDKQG	NLLRNWDVGG	DNLELVDGAY
Consensus	--VR-GSG-Q	F-----	-----	-----	-----V--AY


 — FIGURE 7A —

	251				300
Hap	HYLTAGNTHN	QRGAGNGYSY	LG...D	VRKAGEYGPL	PIAGSKGDSG
HK368IGA	TYGIAGTPYK	VNHENGLIG	FGNSKEEHS	PKGILSQDPL	TNYAVLGDSG
HK393IGA	TYGIAGTPYE	VNHENDGLIG	FGNSNNEYIN	PKEILSKKPL	TNYAVLGDSG
HK715IGA	TYGIAGTPYK	VNHENGLIG	FGNSKEEHS	PKGILSQDPL	TNYAVLGDSG
HK61IGA	TYGIAGTPYK	VNHENGLIG	FGNSKEEHS	PKGILSQDPL	TNYAVLGDSG
Consensus	-Y--AG----	-----G---	-G-----	-----PI	-----GDSG
					*
	301				350
Hap	SPMFIYDAEK	QKWLINGILR	EGNPFEGKEN	GFQLVRKSYF	D.E.IFERDLH
HK368IGA	SPLFVYDREK	GKWLFLGSYD	FWAGYN....KKSQ	EWNIYKSQFT
HK393IGA	SPLFVYDREK	GKWLFLGSYD	YWAGYN....KKSQ	EWNIYKPEFA
HK715IGA	SPLFVYDREK	GKWLFLGSYD	FWAGYN....KKSQ	EWNIYKPEFA
HK61IGA	SPLFVYDREK	GKWLFLGSYD	FWAGYN....KKSQ	EWNIYKHEFA
Consensus	SP-E-YD-EK	-KWL--G---	-----	-----KS--	---I-----
	351				400
Hap	TSLYTRAGNG	VYTISGNDNG	QGSITQKSGI	PSEIKITLAN	MSLPLKEKDK
HK368IGA	KDVLNKDSAG	SLIGSKTDYS	WSSNGKTSTI	TGGEK....S	LNVDLAD...
HK393IGA	EKIYEQYSAG	SLIGSKTDYS	WSSNGKTSTI	TGGEK....S	LNVDLAD...
HK715IGA	KTVLDKDTAG	SLTGSNTQYN	WNPTGKTSVI	SNGSE....S	LNVDLFD...
HK61IGA	EKIYQQYSAG	SLTGSNTQYT	WQATGSTSTI	TGGGE....P	LSVDLTD...
Consensus	-----G	-----S-----	-----S-I	-----	-----L-----
	401				450
Hap	VHNPRYDGN	IYSPRLNNGE	TLYFMDQKQG	SLIFASDINQ	GAGGLYFEGN
HK368IGAGKD.KPNHGK	SVTFEG..SG	TLTLNHNIDQ	GAGGLFFEGD
HK393IGAGKD.KPNHGK	SVTFEG..SG	TLTLNHNIDQ	GAGGLFFEGD
HK715IGASSQD	TDSKKNHGK	SVTLRG..SG	TLTLNHNIDQ	GAGGLFFEGD
HK61IGAGKD.KPNHGK	SITLKG..SG	TLTLNHNIDQ	GAGGLFFEGD
Consensus	-----	-----N-G-	-----G	-L-----I-Q	GAGGL-FEG-
	451				500
Hap	FTVSPNSNQ.	TWQGAGIHVS	ENSTVIWKVN	GVEHDRLSKI	GKGTLHVQAK
HK368IGA	YEVKGTSDNT	TWKGAGVSV	EGKTVIWKVH	NPQYDRLAKI	GKGTLIVEGT
HK393IGA	YEVKGTSDNT	TWKGAGVSV	EGKTVIWKVH	NPQYDRLAKI	GKGTLIVEGT
HK715IGA	YEVKGTSDST	TWKGAGVSV	DGKTVIWKVH	NPKSDRLAKI	GKGTLIVEGK
HK61IGA	YEVKGTSDST	TWKGAGVSV	DGKTVIWKVH	NPKYDRLAKI	GKGTLVVEGK
Consensus	--V---S---	TW-GAG--V-	---TVWKV-	----DRL-KI	GKGTL-V---



— FIGURE 7B —

	501				550
Hap	GENKGSISVG	DGKVILEQQA	DDQGNKQAFS	EIGLVSGRGT	VQLNDDKQFD
HK368IGA	GDNKGSILKVG	DGTVILKQQT	NGSGQ.HAFA	SVGIVSGRST	LVLNDDKQVD
HK393IGA	GDNKGSILKVG	DGTVILKQQT	NGSGQ.HAFA	SVGIVSGRST	LVLNDDKQVD
HK715IGA	GENKGSILKVG	DGTVILKQQA	DANNKVKAFS	QVGIVSGRST	VVLNDDKQVD
HK61IGA	GKNEGLLKVG	DGTVILKQKA	DANNKVQAFS	QVGIVSGRST	LVLNDDKQVD
Consensus	G-N-G--VG	DG-VIL-Q--	-----AF-	--G-VSGR-T	--LNDDKQ-D

	551				600
Hap	TDKFYFGFRG	GRLDLNGHSL	TFKRIQNTDE	GAMIVNHNTT	QAANVTITGN
HK368IGA	PNSIYFGFRG	GRLDLNGNSL	TFDHIRNIDD	GARLVNHMT	NASNITITGE
HK393IGA	PNSIYFGFRG	GRLDLNGNSL	TFDHIRNIDE	GARLVNHSTS	KHSTVTITGD
HK715IGA	PNSIYFGFRG	GRLDANGNL	TFEHIRNIDD	GARLVNHNTS	KTSTVTITGE
HK61IGA	PNSIYFGFRG	GRLDLNGNSL	TFDHIRNIDD	GARVVNHMT	NTSNITITGE
Consensus	----YFGFRG	GRLD-NG--L	TF--I-N-D-	GA--VNH---	-----TITG-

	601				650
Hap	ESIVLPNG..
HK368IGA	SLITDPNTIT	PYNIDAPDED	NPYAFERRIKD	GGQLYLNLEN	YTYVALRKGA
HK393IGA	NLITDPNNVS	IYYVKPLEDD	NPYAIRQIKY	GYQLYFENEEN	RTYYALKKDA
HK715IGA	SLITDPNTIT	PYNIDAPDED	NPYAFERRIKD	GGQLYLNLEN	YTYVALRKGA
HK61IGA	SLITNPNTIT	SYNIEAQDDO	HPLRIRSIPY	R.QLYFNQDN	RSYYTLKKGA
Consensus	--I--PN---	-----	-----	-----	-----

	651				700
HapN	NINKLDYRKE	IAYNGWFGET
HK368IGA	STRSELPKNS	GESNENWLYM	GKTSDEAKRN	VMNHINNERM	NGFNGYFGEE
HK393IGA	SIRSEFPQNR	GESNNSWLYM	GTEKADAQKN	AMNHINNERM	NGFNGYFGEE
HK715IGA	STRSELPKNS	GESNENWLYM	GKTSDEAKRN	VMNHINNERM	NGFNGYFGEE
HK61IGA	STRSELPQNS	GESNENWLYM	GRTSDEAKRN	VMNHINNERM	NGFNGYFGEE
Consensus	-----	-----	-----N	--N-----	---NG-FGE-

	701				750
Hap	D.KNKHNGRL	NLIYKPTTED	RTLLLSGGTN	LKGDITQTKG	KLFFSGRPTP
HK368IGA	EGK..NNGNL	NVTEKKGKSEQ	NRELLTGGTN	LNGDLTVEKG	TLFLSGRPTP
HK393IGA	EGK..NNGNL	NVTEKKGKSEQ	NRELLTGGTN	LNGDLNVQQG	TLFLSGRPTP
HK715IGA	EGK..NNGNL	NVTEKKGKSEQ	NRELLTGGTN	LNGDLKVEKG	TLFLSGRPTP
HK61IGA	ETKATQNGKL	NVTENGKSDQ	NRELLTGGTN	LNGDLNVEKG	TLFLSGRPTP
Consensus	--K--NG-L	N-----	---LL-GGTN	L-GD-----G	-LF-SGRPTP



FIGURE 7C

	751				800
Hap	HAYNHILNKRW	SEMEG..IPQ	GETVWDHDWI	NRTEKAENFQ	IKGGSVVVS.
HK368IGA	HARDLAGISS	TKKDPHEAEN	NEVVVEDDOWI	NRNFKATTMN	VTGNASLYSG
HK393IGA	HARDLAGISS	TKKDSHESEN	NEVVVEDDOWI	NRNFKATNIN	VTNNATLYSG
HK715IGA	HARDLAGISS	TKKDQHEAEN	NEVVVEDDOWI	NRNFKATNIN	VTNNATLYSG
HK61IGA	HARDLAGISS	TKKDPHEAEN	NEVVVEDDOWI	NRNFKATTMN	VTGNASLYSG
Consensus	HA-----	-----	-E-V--DWI	NR-FKA----	-----S-

	801				850
Hap	RNVSSIEGNW	TVSNANATF	GVVFNQNTI	CTRSWTGLT	TCQKVDLDT
HK368IGA	RNVANITSNI	TASNKAQVHI	GY..KTGTV	CVRSDYTGIV	TCTTDKLSD.
HK393IGA	RNVESITSNI	TASNNAKVHI	GY..KAGTV	CVRSDYTGIV	TCTTDKLSD.
HK715IGA	RNVANITSNI	TASDNKVHI	GY..KAGTV	CVRSDYTGIV	TCTTDKLSD.
HK61IGA	RNVANITSNI	TASNKAQVHI	GY..KTGTV	CVRSDYTGIV	TCHNSNLSE.
Consensus	RNV--I--N-	T-S--A----	G-----T-	C-RSD-TG--	TC----L---
				*	*

	851				900
Hap	KVINSIPKTQ	INGSINLTDN	ATANVKGLAK	LNGNVTLTNH	SQFTLSNNAT
HK368IGA	KALNSENPTN	LRGNVNLTES	A.....
HK393IGA	KALNSENPTN	LRGNVNLTES	A.....
HK715IGA	KALNSENATN	VSGNVNLSGN	A.....
HK61IGA	KALNSENPTN	LRGNVNLTEN	A.....
Consensus	K--NS---T-	--G--NL---	A-----	-----	-----

	901				950
Hap	QIGNIRLSDN	STATVDNANL	NGNVHLTDSA	QFSLKNSHFS	HQIQGDKGTT
HK368IGANEVLGKANL	FGTIQSRGNS	QVRLT.....
HK393IGANEVLGKANL	FGTIQSRGNS	QVRLT.....
HK715IGANEVLGKANL	FGTISGIGNS	QVRLT.....
HK61IGASFTLGKANL	FGTIQSIGTS	QVNLK.....
Consensus	-----	-----ANL	-G-----	Q--L-----	-----

	951				1000
Hap	VILENATWIM	PSDTTLQNL	LNNSTITLNS	AYSASSNNTP	RRRSLETETT
HK368IGA	...ENSHWHL	TGNSDVHOLD	LANGHIHLNS	ADNSNNVTK.
HK393IGA	...ENSHWHL	TGNSDVHOLD	LANGHIHLNS	ADNSNNVTK.
HK715IGA	...ENSHWHL	TGDSNVNQLN	LDKGHIHLNA	QNDANKVTT.
HK61IGA	...ENSHWHL	TGNSNVNQLN	LTNGHIHLNA	QNDANKVTT.
Consensus	---EN--W--	-----L-	L----I-LN-	-----	-----

FIGURE 7D

	1001				1050
Hap	PTSAEHRENT	LTVNGKLSGQ	GTFQFTSSLF	GYKSDKLKLS	NDAEGDYILS
HK368IGAYNT	LTVNS.LSGN	GSFYLLTDLS	NKQGDKVVVT	KSATGNFTLQ
HK393IGAYNT	LTVNS.LSGN	GSFYLLTDLS	NKQGDKVVVT	KSATGNFTLQ
HK715IGAYNT	LTVNS.LSGN	GSFYLLTDLS	NKQGDKVVVT	KSATGNFTLQ
HK61IGAYNT	LTVNS.LSGN	GSFYWVDFE	NNKSNKVVVN	KSATGNFTLQ
Consensus	-----NT	LTVN--LSG-	G-F-----	-----K----	--A-G---L-
	1051				1100
Hap	VRNTGKEPET	LEQLTLVESK	DNQPLSDKLK	FTLENDHVDA	GALRYKLVKN
HK368IGA	VADKTGEPNH	.NELTLFDAS	KAQR..DHLN	VSLVGNIVDL	GAWKYKLRNV
HK393IGA	VADKTGEPNH	.NELTLFDAS	KAQR..DHLN	VSLVGNIVDL	GAWKYKLRNV
HK715IGA	VADKTGEPTK	.NELTLFDAS	NATR..NNLN	VSLVGNIVDL	GAWKYKLRNV
HK61IGA	VADKTGEPNH	.NELTLFDAS	NATR..NNLE	VTLANGSVDR	GAWKYKLRNV
Consensus	V-----EP--	---LTL---	-----L-	--L---VD-	GA--YKL---
	1101				1150
Hap	DGEFRLHNP	I KEQELHNDLV
HK368IGA	NGRYDLYNP.	.EVEKRNQTV	DTNITTPNN	IQADVPSVPS	NNEELARVDE
HK393IGA	NGRYDLYNP.	.EVEKRNQTV	DTNITTPNN	IQADVPSVPS	NNEELARVDE
HK715IGA	NGRYDLYNP.	.EVEKRNQTV	DTNITTPNN	IQADVPSVPS	NNEELARV.E
HK61IGA	NGRYDLYNP.	.EVEKRNQTV	DTNITTPND	IQADAPSAQS	NNEELARV.E
Consensus	-G---L-NP-	-E-E--N--V	-----	-----	-----
	1151				1200
Hap
HK368IGA	APVPPPAPAT
HK393IGA	APVPPPAPAT
HK715IGA	TPVPPPAPAT
HK61IGA	TPVPPPAPAT	ESAIASEQPE	TRPAETAQPA	MEETNTANST	ETAPKSDTAT
Consensus	-----	-----	-----	-----	-----
	1201				1250
Hap	RAEQAERTLE	AKQVEPT...
HK368IGA	PSETTETVAE	NSKQESKTVE	KNEQDATETT	AQNREVAKEA
HK393IGA	PSETTETVAE	NSKQESKTVE	KNEQDATETT	AQNREVAKEA
HK715IGA	PSETTETVAE	NSKQESKTVE	KNEQDATETT	AQNGEVAEEA
HK61IGA	QTENPNSES	V PSETTEKVAE	NPPQENETVA	KNEQEATEPT	PQNGEVAKED
Consensus	-----	-----	---Q---T--	-----T---	-----

FIGURE 7E

	1251				1300
HapAKTQT	GE.....
HK368IGA	KSNVKANTQT	NEVAQSGSET	KETQTTETK.ETATVE
HK393IGA	KSNVKANTQT	NEVAQSGSET	KETQTTETK.ETATVE
HK715IGA	KPSVKANTQT	NEVAQSGSET	EETQTTETK.ETAKVE
HK61IGA	QPTVEANTQT	NEATQSEGKT	EETQTAETKS	EPTESVTVSE	NOPEKTVSQS
Consensus	-----A-TQT	-E-----	-----	-----	-----

	1301				1350
Hap
HK368IGA	KEEK.....
HK393IGA	KEEK.....
HK715IGA	KEEKAKVEKE	EKAKVEKDEI	QEPQMASET	SPKQAKPAPK	EVSTDTKVEE
HK61IGA	TEDKVVVEKE	EKAKVETEET	QKAPQVTSKE	PPKQAEPAPE	EVPTDTNAEE
Consensus	-----	-----	-----	-----	-----

	1351				1400
Hap
HK368IGA
HK393IGA
HK715IGA	TQVQAQPQTQ	STTVAAAEAT	SPNSKPAEET	.QPSEKTNAE	PVTPVVSKNQ
HK61IGA	A..QALQQTQ	PTTVAAAEET	SPNSKPAEET	QQPSEKTNAE	PVTPVVS...
Consensus	-----	-----	-----	-----	-----

	1401				1450
HapPKVRS	RRAARAAFPD	TLP.....
HK368IGAAKVETE	KTQEVPKVTS	QVSPKQEQSE	T.....
HK393IGAAKVETE	KTQEVPKVTS	QVSPKQEQSE	T.....
HK715IGA	TENTTDQPT	REKTAKVETE	KTQEPPQVAS	QASPKQEQSE	T.....
HK61IGA	.ENTATQPT	TEETAKVEKE	KTQEVPOVAS	QESPKQEQPA	AKPQAQTKPQ
Consensus	-----	-----	-----P-V-S	-----	-----

	1451				1500
Hap
HK368IGAV
HK393IGAV
HK715IGAV
HK61IGA	AEPARENVL	TKNVGEPQPQ	AQPQTQSTAV	PTTGETAANS	KPAAKPOAQA
Consensus	-----	-----	-----	-----	-----

— **FIGURE 7F** —

	1501			1550	
HapD	QSLINALEA.KQAEI	TAETQKSKAK	TKK.....
HK368IGA	QPQAE	PAREN DPTVNIKEP.QSQTNT	TADTEQPAKE	TSSNVE....
HK393IGA	QPQAE	PAREN DPTVNIKEP.QSQTNT	TADTEQPAKE	TSSNVE....
HK715IGA	QPQAV	LESEN VPTVNNAEEV	QAQLQTQ	TSATVSTKQ	PAPENSINTG....
HK61IGA	KPQTE	PAREN VSTVNTKEP.QSQ	TSATVSTKQ	PAPENSINTG....
Consensus	-----	---N-E-	-----Q-	T--T-----	-----

	1551			1600	
HapV	RSKRAVESDP	LLDQSL....
HK368IGAQPVT	ESTTVNTGNS	VVEN.....
HK393IGAQPVT	ESTTVNTGNS	VVEN.....
HK715IGASAT	AITETAEKSD	KPQTETA	ASTEDASQ	HKANTVADNSVANNS
HK61IGA	ENSINTGSAT	TMTETAEKSD	KPQMET..VT	ENDROPEANT	VADNSVANNS
Consensus	-----	-----	-----	-----	-----

	1601			1650	
HapF	ALEAALEVID	APQQSEK	DRLAQEEAEKQ
HK368IGA	PENTTPATTQ	PTVNSESSN.	.KPK.NRHR
HK393IGA	PENTTPATTQ	PTVNSESSN.	.KPK.NRHR
HK715IGA	ESSEPKSRRR	RSISQPQETS	AEETTAASTD	ETTIADNSKR	SKPN.RRSRR
HK61IGA	ESSESRSRRR	RSVSQPKETS	AEETTIVASTQ	ETTVDNSVST	PKPRSRRRTR
Consensus	-----	-----	-----	-----	-----R-

	1651			1700	
HapQKDLI	SRYSNSALSE	
HK368IGA	SVRSVPHNVE	PATTSSND..	RSTVALCDLT	STNTNAVLSD
HK393IGA	SVRSVPHNVE	PATTSSND..	RSTVALCDLT	STNTNAVLSD
HK715IGA	SVRS.....E	PTVINGSD..	RSTVALRDLT	STNTNAVISD
HK61IGA	SVQTNSYEPV	ELPTENAENA	ENVQSGNVA	NSQPALRNLT	SKNTNAVLSN
Consensus	-----	-----	-----	-----L-	S---N---S-

	1701			1750	
Hap	LSA.....TV	NSMLSVQDEL	DRL.FVDQAO	SAVWTNIAQD	KRRYDSDAFR
HK368IGA	ARAKAQEVAL	NVGKAVSQHI	SOLEMNNEGQ	YNVWVSNTSM	NKNYSSSQYR
HK393IGA	ARAKAQEVAL	NVGKAVSQHI	SOLEMNNEGQ	YNVWVSNTSM	NKNYSSSQYR
HK715IGA	AMAKAQEVAL	NVGKAVSQHI	SOLEMNNEGQ	YNVWVSNTSM	NENYSSSQYR
HK61IGA	AMAKAQEVAL	NVGKAVSQHI	SOLEMNNEGQ	YNVWISNTSM	NKNYSSEQYR
Consensus	--A-----	N---V----	--L-----Q	--VW-----	---Y-S---R



FIGURE 7G

	1751		1800
Hap	AYQQQKINLR	QIGVQKALAN	GRIGAVFSHS RSDNTEDEQV KNHATLIMMS
HK368IGA	RESSKSTQTQ	LGWDQTISSN	VQLGGVFTYV RNSNNEDKAT SKN.TLAQVN
HK393IGA	RESSKSTQTQ	LGWDQTISSN	VQLGGVFTYV RNSNNEDKAT SKN.TLAQVN
HK715IGA	RESSKSTQTQ	LGWDQTISSN	VQLGGVFTYV RNSNNEDKAS SKN.TLAQVN
HK61IGA	RESSKSTQTQ	LGWDQTISSN	VQLGGVFTYV RNSNNEDKAS SKN.TLAQVN
Consensus	-----T-----	-----Q-----N	---G-VF--- R--N-FD--- ----TL----

	1801		1850
Hap	GFAQYQWGL	QF..GVNVGT	GISASKMAEE QSRKIHRKAI NYGVNASYQF
HK368IGA	FYSKY.YADN	HWYLGIDLGY	GKFQSKLQTN HNAKFARHTA QFGLTAGKAF
HK393IGA	FYSKY.YADN	HWYLGIDLGY	GKFQSKLQTN HNAKFARHTA QFGLTAGKAF
HK715IGA	FYSKY.YADN	HWYLGIDLGY	GKFQSNLKTN HNAKFARHTA QFGLTAGKAF
HK61IGA	FYSKY.YADN	HWYLGIDLGY	GKFQSNLQTN HNAKFARHTA QIGLTAGKAF
Consensus	----Y--D-	----G--G-	G---S----- ----K--R--- --G--A---F

	1851		1900
Hap	RLGQLGIQPY	FGVNRVYFIER	ENYQSEEV RV KTPSLAENRY NAGIRVDYTF
HK368IGA	NLGNFGITPI	VGVRYSYLSN	ADFALDQARI KVNPI SVKTA FAQVDLSYTY
HK393IGA	NLGNFGITPI	VGVRYSYLSN	ADFALDQARI KVNPI SVKTA FAQVDLSYTY
HK715IGA	NLGNFGITPI	VGVRYSYLSN	ANFALAKDRI KVNPI SVKTA FAQVDLSYTY
HK61IGA	NLGNFAVKPT	VGVRYSYLSN	ADFALAQDRI KVNPI SVKTA FAQVDLSYTY
Consensus	-LG-----P-	-GV-----	-----R- K----- -A-----YT-

	1901		1950
Hap	TPTDNLSVKP	YFEVNYVDVS	NANVQTTVNL TVLQQPFGRY WQKEVGLKAE
HK368IGA	.HLGEFSVTP	ILSARY.DAN	QSGSKINVNG YDFAYNVENQ QQYNAGLKLK
HK393IGA	.HLGEFSVTP	ILSARY.DAN	QSGSKINVNG YDFAYNVENQ QQYNAGLKLK
HK715IGA	.HLGEFSVTP	ILSARY.DTN	QSGSKINVNQ YDFAYNVENQ QQYNAGLKLK
HK61IGA	.HLGEFSITP	ILSARY.DAN	QGNKINVSU YDFAYNVENQ QQYNAGLKLK
Consensus	-----S--P	-----Y-D--	-----V-- ----- -Q---GLK--

	1951		1982
Hap	ILHFQISAFI	SKSQGSQLGK	QONVGVKLG Y RW
HK368IGA	YHNVKLSLIG	GLTKAKQAEK	OKTAELKLSF SF
HK393IGA	YHNVKLSLIG	GLTKAKQAEK	OKTAELKLSF SF
HK715IGA	YHNVKLSLIG	GLTKAKQAEK	OKTAELKLSF SF
HK61IGA	YHNVKLSLIG	GLTKAKQAEK	OKTAEVKLSF SF
Consensus	-----S---	-----Q--K	Q-----KL-- --




 FIGURE 7H

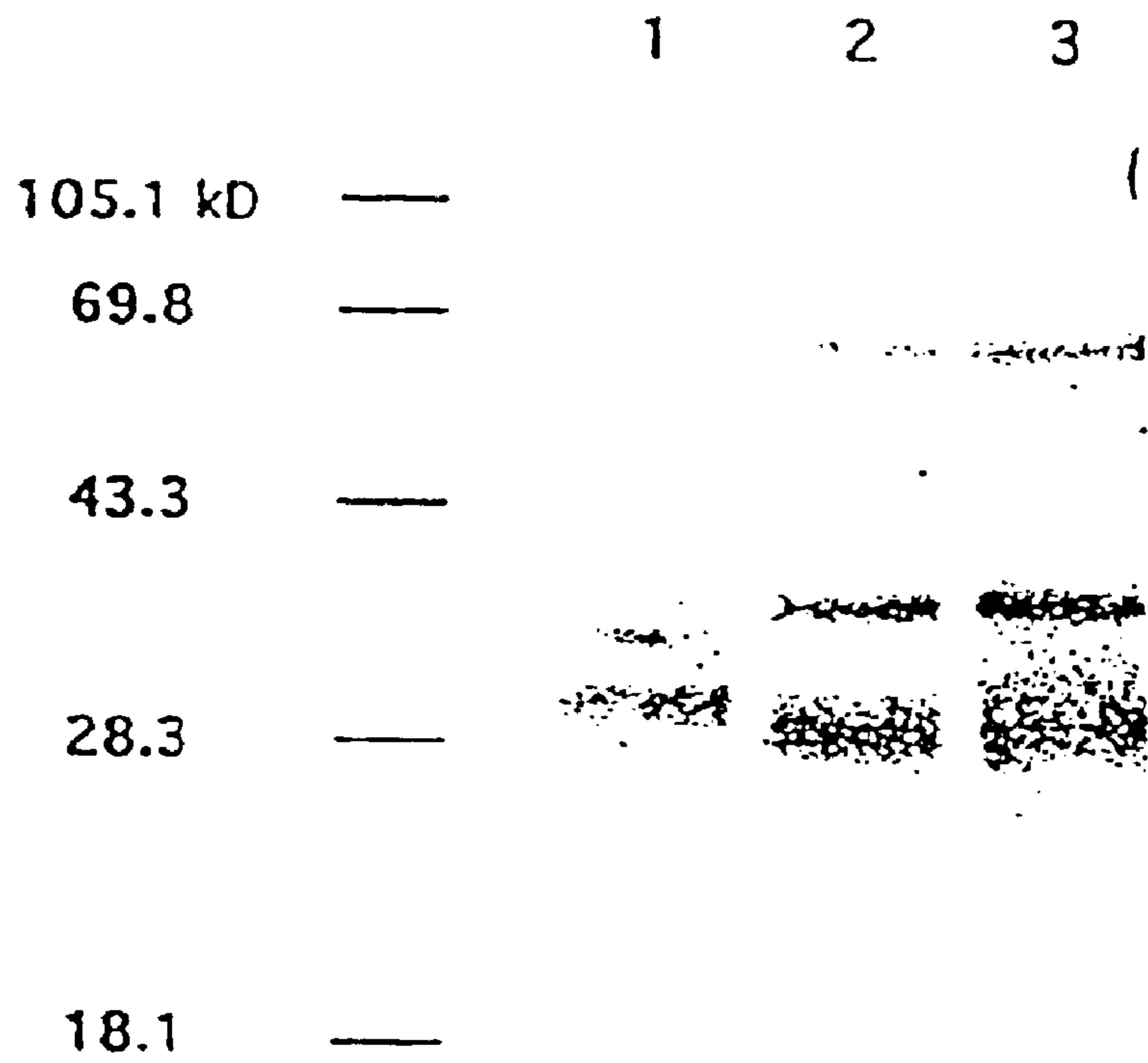
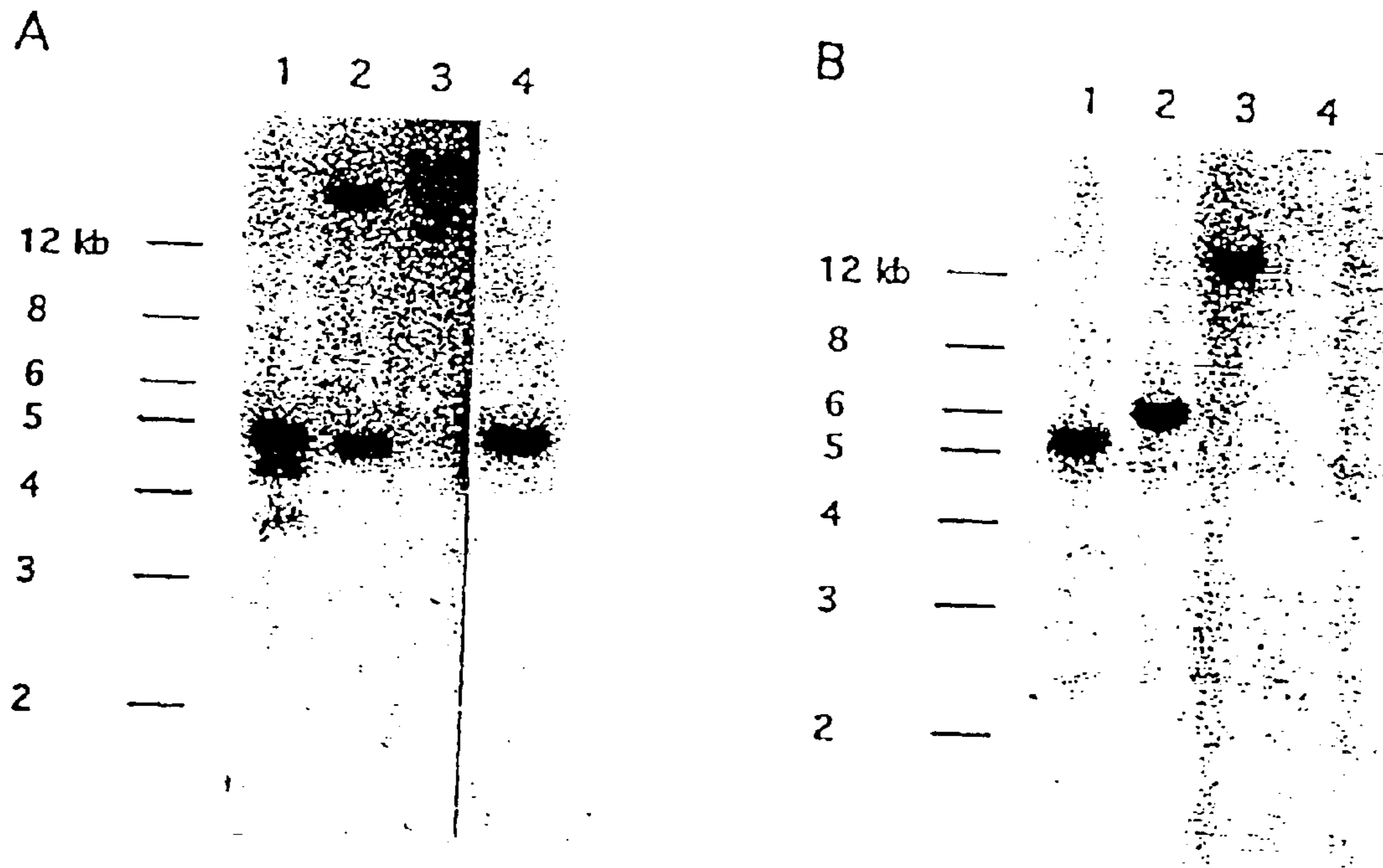


FIGURE 8



— **FIGURE 9** —

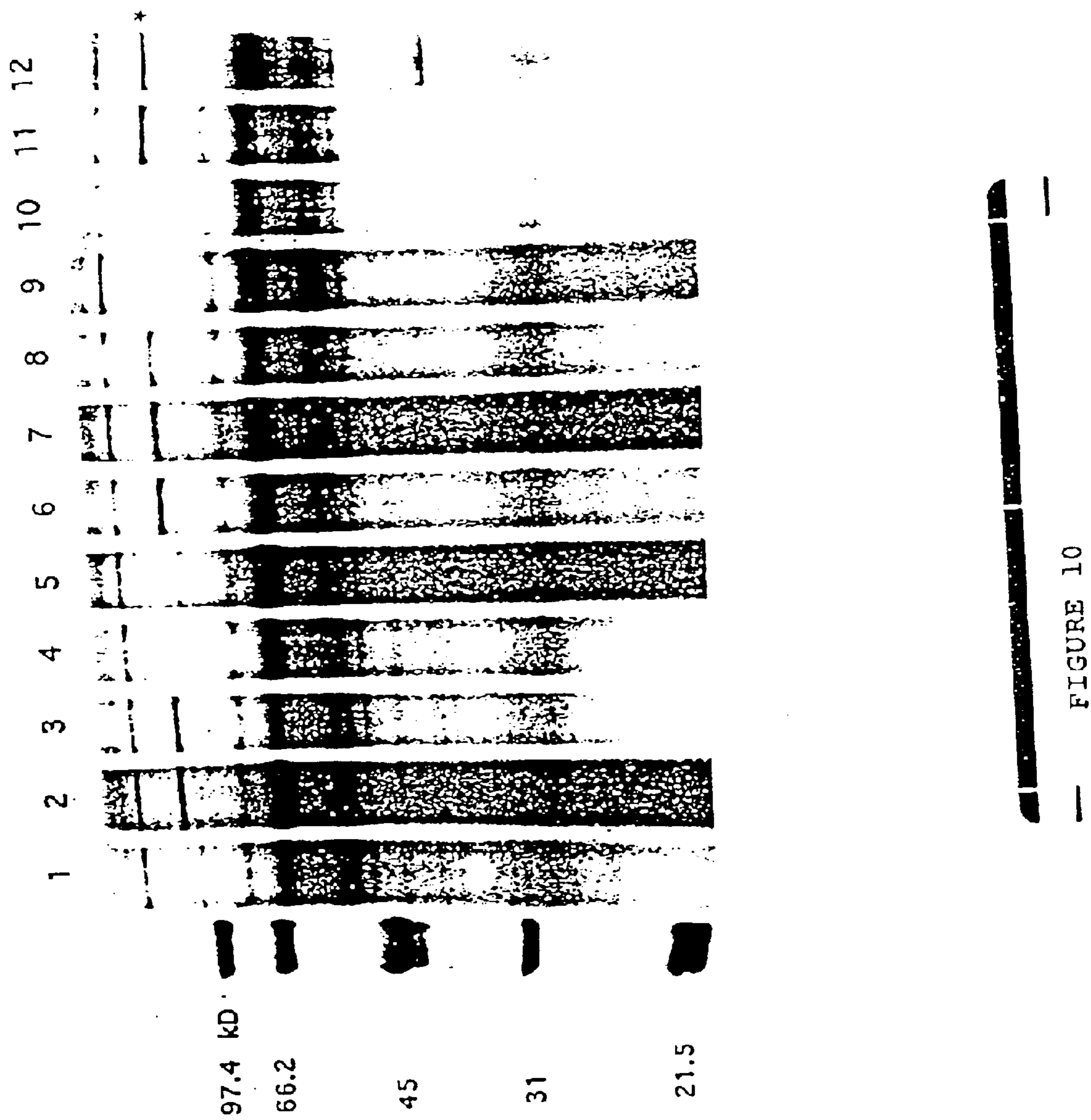


FIGURE 10

HAEMOPHILUS ADHERENCE AND PENETRATION PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a divisional of U.S. Ser. No. 09/839,996, filed Apr. 20, 2001, now U.S. Pat. No. 6,642,371, which is a divisional of U.S. Ser. No. 08/296,791, filed Aug. 25, 1994, now U.S. Pat. No. 6,245,337.

This invention was made with government support under grant numbers HD 29678 and AI 23945 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

The invention relates to Haemophilus adhesion and penetration proteins, nucleic acids, and vaccines.

BACKGROUND OF THE INVENTION

Most bacterial diseases begin with colonization of a particular mucosal surface (Beachey et al., 1981, J. Infect. Dis. 143:325–345). Successful colonization requires that an organism overcome mechanical cleansing of the mucosal surface and evade the local immune response. The process of colonization is dependent upon specialized microbial factors that promote binding to host cells (Hultgren et al., 1993 Cell, 73:887–901). In some cases the colonizing organism will subsequently enter (invade) these cells and survive intracellularly (Falkow, 1991, Cell 65:1099–1102).

Haemophilus influenzae is a common commensal organism of the human respiratory tract (Kuklinska and Kilian, 1984, Eur. J. Clin. Microbiol. 3:249–252). It is a human-specific organism that normally resides in the human nasopharynx and must colonize this site in order to avoid extinction. This microbe has a number of surface structures capable of promoting attachment to host cells (Guerina et al., 1982, J. Infect. Dis. 146:564; Pichichero et al., 1982, Lancet ii:960–962; St. Geme et al., 1993, Proc. Natl. Acad. Sci. U.S.A. 90:2875–2879). In addition, *H. influenzae* has acquired the capacity to enter and survive within these cells (Forsgren et al., 1994, Infect. Immun. 62:673–679; St. Geme and Falkow, 1990, Infect. Immun. 58:4036–4044; St. Geme and Falkow, 1991, Infect. Immun. 59:1325–1333, Infect. Immun. 59:3366–3371). As a result, this bacterium is an important cause of both localized respiratory tract and systemic disease (Turk, 1984, J. Med. Microbiol. 18:1–16). Nonencapsulated, non-typable strains account for the majority of local disease (Turk, 1984, supra); in contrast, serotype b strains, which express a capsule composed of a polymer of ribose and ribitol-5-phosphate (PRP), are responsible for over 95% of cases of *H. influenzae* systemic disease (Turk, 1982, Clinical importance of *Haemophilus influenzae*, p. 3–9. In S. H. Sell and P. F. Wright (ed.), *Haemophilus influenzae* epidemiology, immunology, and prevention of disease. Elsevier/North-Holland Publishing Co., New York).

The initial step in the pathogenesis of disease due to *H. influenzae* involves colonization of the upper respiratory mucosa (Murphy et al., 1987, J. Infect. Dis. 5:723–731). Colonization with a particular strain may persist for weeks to months, and most individuals remain asymptomatic throughout this period (Spinola et al., 1986, I. Infect. Dis. 154:100–109). However, in certain circumstances colonization will be followed by contiguous spread within the respiratory tract, resulting in local disease in the middle ear, the sinuses, the conjunctiva, or the lungs. Alternatively, on

occasion bacteria will penetrate the nasopharyngeal epithelial barrier and enter the bloodstream.

In vitro observations and animal studies suggest that bacterial surface appendages called pili (or fimbriae) play an important role in *H. influenzae* colonization. In 1982 two groups reported a correlation between piliation and increased attachment to human oropharyngeal epithelial cells and erythrocytes (Guerina et al., supra; Pichichero et al., supra). Other investigators have demonstrated that anti-pilus antibodies block in vitro attachment by piliated *H. influenzae* (Forney et al., 1992, J. Infect. Dis. 165:464–470; van Alphen et al., 1988, Infect. Immun. 56:1800–1806). Recently Weber et al. insertionally inactivated the pilus structural gene in an *H. influenzae* type b strain and thereby eliminated expression of pili; the resulting mutant exhibited a reduced capacity for colonization of year-old monkeys (Weber et al., 1991, Infect. Immun. 59:4724–4728).

A number of reports suggest that nonpilus factors also facilitate Haemophilus colonization. Using the human nasopharyngeal organ culture model, Farley et al. (1986, J. Infect. Dis. 161:274–280) and Loeb et al. (1988, Infect. Immun. 49:484–489) noted that nonpiliated type b strains were capable of mucosal attachment. Read and coworkers made similar observations upon examining nontypable strains in a model that employs nasal turbinate tissue in organ culture (1991, J. Infect. Dis. 163:549–558). In the monkey colonization study by Weber et al. (1991, supra), nonpiliated organisms retained a capacity for colonization, though at reduced densities; moreover, among monkeys originally infected with the piliated strain, virtually all organisms recovered from the nasopharynx were nonpiliated. All of these observations are consistent with the finding that nasopharyngeal isolates from children colonized with *H. influenzae* are frequently nonpiliated (Mason et al., 1985, Infect. Immun. 49:98–103; Brinton et al., 1989, Pediatr. Infect. Dis. J. 8:554–561).

Previous studies have shown that *H. influenzae* are capable of entering (invading) cultured human epithelial cells via a pili-independent mechanism (St. Geme and Falkow, 1990, supra; St. Geme and Falkow, 1991, supra). Although *H. influenzae* is not generally considered an intracellular parasite, a recent report suggests that these in vitro findings may have an in vivo correlate (Forsgren et al., 1994, supra). Forsgren and coworkers examined adenoids from 10 children who had their adenoids removed because of long-standing secretory otitis media or adenoidal hypertrophy. In all 10 cases there were viable intracellular *H. influenzae*. Electron microscopy demonstrated that these organisms were concentrated in the reticular crypt epithelium and in macrophage-like cells in the subepithelial layer of tissue. One possibility is that bacterial entry into host cells provides a mechanism for evasion of the local immune response, thereby allowing persistence in the respiratory tract.

Thus, a vaccine for the therapeutic and prophylactic treatment of Haemophilus infection is desirable. Accordingly, it is an object of the present invention to provide for recombinant Haemophilus Adherence and Penetration (HAP) proteins and variants thereof, and to produce useful quantities of these HAP proteins using recombinant DNA techniques.

It is a further object of the invention to provide recombinant nucleic acids encoding HAP proteins, and expression vectors and host cells containing the nucleic acid encoding the HAP protein.

An additional object of the invention is to provide monoclonal antibodies for the diagnosis of Haemophilus infection.

A further object of the invention is to provide methods for producing the HAP proteins, and a vaccine comprising the HAP proteins of the present invention. Methods for the therapeutic and prophylactic treatment of *Haemophilus* infection are also provided.

SUMMARY OF THE INVENTION

In accordance with the foregoing objects, the present invention provides recombinant HAP proteins, and isolated or recombinant nucleic acids which encode the HAP proteins of the present invention. Also provided are expression vectors which comprise DNA encoding a HAP protein operably linked to transcriptional and translational regulatory DNA, and host cells which contain the expression vectors.

The invention provides also provides methods for producing HAP proteins which comprises culturing a host cell transformed with an expression vector and causing expression of the nucleic acid encoding the HAP protein to produce a recombinant HAP protein.

The invention also includes vaccines for *Haemophilus influenzae* infection comprising an HAP protein for prophylactic or therapeutic use in generating an immune response in a patient. Methods of treating or preventing *Haemophilus influenzae* infection comprise administering a vaccine.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B depict light micrographs of *H. influenzae* strains DB117(pGJB103) and DB117(pN187) incubated with Chang epithelial cells. Bacteria were incubated with an epithelial monolayer for 30 minutes before rinsing and straining with Giemsa stain. FIG. 1A: *H. influenzae* strain DB117 carrying cloning vector alone (pGJB103); FIG. 1B: *H. influenzae* strain DB117 harboring recombinant plasmid pH187. Bar represents 3.5 μm .

FIGS. 2A, 2B, 2C and 2D depict thin section transmission electron micrographs demonstrating interaction between *H. influenzae* strains N187 and DB117(pN187) with Chang epithelial cells. Bacteria were incubated with epithelial monolayers for four hours before rinsing and processing for examination by transmission electron microscopy. FIG. 2A: strain N187 associated with the epithelial cell surface and present in an intracellular location; FIG. 2B: *H. influenzae* DB117 (pH187) in intimate contact with the epithelial cell surface; FIG. 2C: strain DB117(pN187) in the process of entering an epithelial cell; FIG. 2D: strain DB117(pN187) present in an intracellular location. Bar represents 1 μm .

FIG. 3 depicts outer membrane protein profiles of various strains. Outer membrane proteins were isolated on the basis of sarcosyl insolubility and resolved on a 10% SDS-polyacrylamide gel. Proteins were visualized by staining with Coomassie blue. Lane 1, *H. influenzae* strain DB117 (pGJB103); lane 2, strain DB117(pN187); lane 3, strain DB117(pJS106); lane 4, *E. coli* HB101(pGJB103); lane 5, HB101(pN187). Note novel proteins at -160 kD and 45 kD marked by asterisks in lanes 2 and 3.

FIG. 4 depicts a restriction map of pN187 and derivatives and locations of mini-Tn10 kan insertions. pN187 is a derivative of pGJB103 that contains an 8.5-kb Sau3AI fragment of chromosomal DNA from *H. influenzae* strain N187. Vector sequences are represented by hatched boxes. Letters above top horizontal line indicate restriction enzyme sites: Bg, BgIII; C, ClaI; E, EcoRI; P, PstI. Numbers and lollipops above top horizontal line show positions of mini-Tn10 kan insertions; open lollipops represent insertions that

have no effect on adherence and invasion, while closed lollipops indicate insertions that eliminate the capacity of pN187 to promote association with epithelial monolayers. Heavy horizontal line with arrow represents location of hap locus within pN187 and direction of transcription. (+): recombinant plasmids that promote adherence and invasion; (-): recombinant plasmids that fail to promote adherence and invasion.

FIG. 5 depicts the identification of plasmid-encoded proteins using the bacteriophage T7 expression system. Bacteria were radiolabeled with [^{35}S] methionine, and whole cell lysates were resolved on a 10% SDS-polyacrylamide gel. Proteins were visualized by autoradiography. Lane 1, *E. coli* XL-1 Blue(pT7-7) uninduced; lane 2, XL-1 Blue(pT7-7) induced with IPTG; lane 3, XL-1 Blue(pJS103) uninduced; lane 4, XL-1 Blue(pJS103) induced with IPTG; lane 5, XL-1 Blue(pJS104) uninduced; lane 6, XL-1 Blue(pJS104) induced with IPTG. The plasmids pJS103 and pJS104 are derivatives of pT7-7 that contain the 6.5-kb PstI fragment from pN187 in opposite orientations. Asterisk indicates overexpressed protein in XL-1 Blue(pJS104).

FIGS. 6A, 6B, and 6C depict the nucleotide sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) of hap gene. Putative -10 and -35 sequences 5' to the hap coding sequence are underlined; a putative rho-independent terminator 3' to the hap stop codon is indicated with inverted arrows. The first 25 amino acids of the protein, which are boxed, represent the signal sequence.

FIGS. 7A, 7B, 7C, 7D, 7E, 7F, 7G, and 7H depict a sequence comparison of the hap product and the cloned *H. influenzae* IgA1 proteases. Amino acid homologies between the deduced hap gene product and the iga gene products from *H. influenzae* HK368 (SEQ ID NO:3) HK61 (SEQ ID NO:6), HK393 (SEQ ID NO:4), and HK793 (SEQ ID NO:5) are shown. Dashes indicate gaps introduced in the sequences in order to obtain maximal homology. A consensus sequence for the five proteins is shown on the lower line. The conserved serine-type protease catalytic domain is underlined, and the common active site serine is denoted by an asterisk. The conserved cysteines are also indicated by asterisks.

FIG. 8 depicts the IgA1 protease activity assay. Culture supernatants were assayed for the ability to cleave IgA1. Reaction mixtures were resolved on a 10% SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane. The membrane was probed with antibody against human IgA1 heavy chain. Lane 1, *H. influenzae* strain N187; lane 2, strain DB117(pGJB103); lane 3, strain DB117(pN187). The cleavage product patterns suggest that strain N187 contains a type 2 IgA1 protease while strains DB117(pGJB103) and DB117(pN187) contain a type 1 enzyme. The upper band of -70-kD seen with the DB117 derivatives represents intact IgA1 heavy chain.

FIGS. 9A and 9B depict southern analysis of chromosomal DNA from strain *H. influenzae* N187, probing with hap versus iga. DNA fragments were separated on a 0.7% agarose gel and transferred bidirectionally to nitrocellulose membranes prior to probing with either hap or iga. Lane 1, N187 chromosomal DNA digested with EcoRI; lane 2, N187 chromosomal DNA digested with BglII; lane 3, N187 chromosomal DNA digested with BamHI; lane 4, the 4.8-kb ClaI-PstI fragment from pN187 that contains the intact hap gene. FIG. 9A: Hybridization with the 4.8-kb ClaI-PstI fragment containing the hap gene; FIG. 9B: hybridization with the iga gene from *H. influenzae* strain Rd, carried as a 4.8-kb ClaI-EcoRI fragment in pVD116.

FIG. 10 depicts a SDS-polyacrylamide gel of secreted proteins. Bacteria were grown to late log phase, and culture supernatants were precipitated with trichloroacetic acid and then resolved on a 10% SDS-polyacrylamide gel. Proteins were visualized by staining with Coomassie blue. Lane 1, *H. influenzae* strain DB117(pGJB103); lane 2, DB117(pN187); lane 3, DB117(pJS106); lane 4, DB117(pJS102); lane 5, DB117(pJS105); lane 6, DB117(Tn10-18); lane 7, DB117(Tn10-4'); lane 8, DB117(Tn10-30); lane 9, DB117(Tn10-16); lane 10, DB117(Tn10-10); lane 11, DB117(Tn10-8); lane 12, N187. Asterisk indicates 110-kD secreted protein encoded by hap.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel *Haemophilus* Adhesion and Penetration (HAP) proteins. In a preferred embodiment, the HAP proteins are from *Haemophilus* strains, and in the preferred embodiment, from *Haemophilus influenzae*. However, using the techniques outlined below, HAP proteins from other *Haemophilus influenzae* strains, or from other bacterial species such as *Neisseria* spp. or *Bordetella* spp. may also be obtained.

A HAP protein may be identified in several ways. A HAP nucleic acid or HAP protein is initially identified by substantial nucleic acid and/or amino acid sequence homology to the sequences shown in FIG. 6. Such homology can be based upon the overall nucleic acid or amino acid sequence.

The HAP proteins of the present invention have limited homology to *Haemophilus influenzae* and *N. gonorrhoeae* serine-type IgA1 proteases. This homology, shown in FIG. 7, is approximately 30–35% at the amino acid level, with several stretches showing 55–60% identity, including amino acids 457–549, 399–466, 572–622, and 233–261. However, the homology between the HAP protein and the IgA1 protease is considerably lower than the similarity among the IgA1 proteases themselves.

In addition, the full length HAP protein has homology to Tsh, a hemagglutinin expressed by an avian *E. coli* strain (Provence and Curtiss 1994, *Infect. Immun.* 62:1369–1380). The homology is greatest in the N-terminal half of the proteins, and the overall homology is 30.5% homologous. The full length HAP protein also has homology with pertactin, a 69 kD outer membrane protein expressed by *B. pertussis*, with the middle portion of the proteins showing 39% homology. Finally, HAP has 34–52% homology with six regions of HpmA, a calcium-independent heinolysin expressed by *Proteus mirabilis* (Uphoff and Welch, 1990, *J. Bacteriol.* 172:1206–1216).

As used herein, a protein is a “HAP protein” if the overall homology of the protein sequence to the amino acid sequence shown in FIG. 6 (SEQ ID NO:2) is preferably greater than about 40–50%, more preferably greater than about 60% and most preferably greater than 80%. In some embodiments the homology will be as high as about 90 to 95 or 98%. This homology will be determined using standard techniques known in the art, such as the Best Fit sequence program described by Devereux et al, *Nucl. Acid Res.* 12:387–395 (1984). The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer amino acids than the protein shown in FIG. 6, it is understood that the percentage of homology will be determined based on the number of homologous amino acids in relation to the total number of amino acids. Thus, for example, homology of sequences shorter than that shown in FIG. 6, as discussed

below, will be determined using the number of amino acids in the shorter sequence.

HAP proteins of the present invention may be shorter than the amino acid sequence shown in FIG. 6. As shown in the Examples, the HAP protein may undergo post-translational processing similar to that seen for the serine-type IgA1 proteases expressed by *Haemophilus influenzae* and *N. gonorrhoeae*. These proteases are synthesized as preproteins with three functional domains: the N-terminal signal peptide, the protease, and a C-terminal helper domain. Following movement of these proteins into the periplasmic space, the carboxy terminal β -domain of the proenzyme is inserted into the outer membrane, possibly forming a pore (Poulsen et al., 1989, *Infect. Immun.* 57:3097–3105; Pohlner et al., 1987, *Nature (London)*. 325:458–462; Klauser et al., 1992, *EMBO J.* 11:2327–2335; Klauser et al., 1993, *J. Mol. Biol.* 234:579–593). Subsequently the amino end of the protein is exported through the outer membrane, and auto-proteolytic cleavage occurs to result in secretion of the mature 100 to 106-kD protease. The 45 to 56-kD C-terminal β -domain remains associated with the outer membrane following the cleavage event. As shown in the Examples, the HAP nucleic acid is associated with expression of a 160 kD outer membrane protein. The secreted gene product is an approximately 110 kD protein, with the simultaneous appearance of a 45 kD outer membrane protein. The 45 kD protein appears to correspond to amino acids from about 960 to about 1394 of FIG. 6. Any one of these proteins is considered a HAP protein for the purposes of this invention.

Thus, in a preferred embodiment, included within the definition of HAP proteins are portions or fragments of the sequence shown in FIG. 6. The fragments may be fragments of the entire sequence, the 110 kD sequence, or the 45 kD sequence. Generally, the HAP protein fragments may range in size from about 10 amino acids to about 1900 amino acids, with from about 50 to about 1000 amino acids being preferred, and from about 100 to about 500 amino acids also preferred. Particularly preferred fragments are sequences unique to HAP; these sequences have particular use in cloning HAP proteins from other organisms or to generate antibodies specific to HAP proteins. Unique sequences are easily identified by those skilled in the art after examination of the HAP protein sequence and comparison to other proteins; for example, by examination of the sequence alignment shown in FIG. 7. For instance, as compared to the IgA proteases, unique sequences include, but are not limited to, amino acids 11–14, 16–22, 108–120, 155–164, 257–265, 281–288, 318–336, 345–353, 398–416, 684–693, 712–718, 753–761, 871–913, 935–953, 985–1008, 1023–1034, 1067–1076, 1440–1048, 1585–1592, 1631–1639, 1637–1648, 1735–1743, 1863–1871, 1882–1891, 1929–1941, and 1958–1966 (using the numbering of FIG. 7). HAP protein fragments which are included within the definition of a HAP protein include N- or C-terminal truncations and deletions which still allow the protein to be biologically active; for example, which still exhibit proteolytic activity in the case of the 110 kD putative protease sequence. In addition, when the HAP protein is to be used to generate antibodies, for example as a vaccine, the HAP protein must share at least one epitope or determinant with either the full length protein, the 110 kD protein or the 45 kD protein, shown in FIG. 6. In a preferred embodiment, the epitope is unique to the HAP protein; that is, antibodies generated to a unique epitope exhibit little or no cross-reactivity with other proteins. By “epitope” or “determinant” herein is meant a portion of a protein which will generate and/or bind an antibody. Thus, in most instances, antibodies made to a smaller HAP protein will be able to bind to the full length protein.

In some embodiments, the fragment of the HAP protein used to generate antibodies are small; thus, they may be used as haptens and coupled to protein carriers to generate antibodies, as is known in the art.

Preferably, the antibodies are generated to a portion of the HAP protein which remains attached to the *Haemophilus influenzae* organism. For example, the HAP protein can be used to vaccinate a patient to produce antibodies which upon exposure to the *Haemophilus influenzae* organism (e.g. during a subsequent infection) bind to the organism and allow an immune response. Thus, in one embodiment, the antibodies are generated to the roughly 45 kD fragment of the full length HAP protein. Preferably, the antibodies are generated to the portion of the 45 kD fragment which is exposed at the outer membrane.

In an alternative embodiment, the antibodies bind to the mature secreted 110 kD fragment. For example, as explained in detail below, the HAP proteins of the present invention may be administered therapeutically to generate neutralizing antibodies to the 110 kD putative protease, to decrease the undesirable effects of the 100 kD fragment.

In the case of the nucleic acid, the overall homology of the nucleic acid sequence is commensurate with amino acid homology but takes into account the degeneracy in the genetic code and codon bias of different organisms. Accordingly, the nucleic acid sequence homology may be either lower or higher than that of the protein sequence. Thus the homology of the nucleic acid sequence as compared to the nucleic acid sequence of FIG. 6 is preferably greater than 40%, more preferably greater than about 60% and most preferably greater than 80%. In some embodiments the homology will be as high as about 90 to 95 or 98%.

In one embodiment, the nucleic acid homology is determined through hybridization studies. Thus, for example, nucleic acids which hybridize under high stringency to all or part of the nucleic acid sequence shown in FIG. 6 are considered HAP protein genes. High stringency conditions include washes with 0.1×SSC at 65° C. for 2 hours.

The HAP proteins and nucleic acids of the present invention are preferably recombinant. As used herein, “nucleic acid” may refer to either DNA or RNA, or molecules which contain both deoxy- and ribonucleotides. The nucleic acids include genomic DNA, cDNA and oligonucleotides including sense and anti-sense nucleic acids. Specifically included within the definition of nucleic acid are anti-sense nucleic acids. An anti-sense nucleic acid will hybridize to the corresponding non-coding strand of the nucleic acid sequence shown in FIG. 6, but may contain ribonucleotides as well as deoxyribonucleotides. Generally, anti-sense nucleic acids function to prevent expression of mRNA, such that a HAP protein is not made, or made at reduced levels. The nucleic acid may be double stranded, single stranded, or contain portions of both double stranded or single stranded sequence. By the term “recombinant nucleic acid” herein is meant nucleic acid, originally formed in vitro by the manipulation of nucleic acid by endonucleases, in a form not normally found in nature. Thus an isolated HAP protein gene, in a linear form, or an expression vector formed in vitro by ligating DNA molecules that are not normally joined, are both considered recombinant for the purposes of this invention. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e. using the in vivo cellular machinery of the host cell rather than in vitro manipulations; however, such nucleic acids, once produced recombinantly, although subsequently replicated non-

recombinantly, are still considered recombinant for the purposes of the invention.

Similarly, a “recombinant protein” is a protein made using recombinant techniques, i.e. through the expression of a recombinant nucleic acid as depicted above. A recombinant protein is distinguished from naturally occurring protein by at least one or more characteristics. For example, the protein may be isolated away from some or all of the proteins and compounds with which it is normally associated in its wild type host, or found in the absence of the host cells themselves. Thus, the protein may be partially or substantially purified. The definition includes the production of a HAP protein from one organism in a different organism or host cell. Alternatively, the protein may be made at a significantly higher concentration than is normally seen, through the use of a inducible promoter or high expression promoter, such that the protein is made at increased concentration levels. Alternatively, the protein may be in a form not normally found in nature, as in the addition of an epitope tag or amino acid substitutions, insertions and deletions.

Also included with the definition of HAP protein are HAP proteins from other organisms, which are cloned and expressed as outlined below.

In the case of anti-sense nucleic acids, an anti-sense nucleic acid is defined as one which will hybridize to all or part of the corresponding non-coding sequence of the sequence shown in FIG. 6. Generally, the hybridization conditions used for the determination of anti-sense hybridization will be high stringency conditions, such as 0.1×SSC at 65° C.

Once the HAP protein nucleic acid is identified, it can be cloned and, if necessary, its constituent parts recombined to form the entire HAP protein nucleic acid. Once isolated from its natural source, e.g., contained within a plasmid or other vector or excised therefrom as a linear nucleic acid segment, the recombinant HAP protein nucleic acid can be further used as a probe to identify and isolate other HAP protein nucleic acids. It can also be used as a “precursor” nucleic acid to make modified or variant HAP protein nucleic acids and proteins.

Using the nucleic acids of the present invention which encode HAP protein, a variety of expression vectors are made. The expression vectors may be either self-replicating extrachromosomal vectors or vectors which integrate into a host genome. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the HAP protein. “Operably linked” in this context means that the transcriptional and translational regulatory DNA is positioned relative to the coding sequence of the HAP protein in such a manner that transcription is initiated. Generally, this will mean that the promoter and transcriptional initiation or start sequences are positioned 5' to the HAP protein coding region. The transcriptional and translational regulatory nucleic acid will generally be appropriate to the host cell used to express the HAP protein; for example, transcriptional and translational regulatory nucleic acid sequences from *Bacillus* will be used to express the HAP protein in *Bacillus*. Numerous types of appropriate expression vectors, and suitable regulatory sequences are known in the art for a variety of host cells.

In general, the transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator

sequences. In a preferred embodiment, the regulatory sequences include a promoter and transcriptional start and stop sequences.

Promoter sequences encode either constitutive or inducible promoters. The promoters may be either naturally occurring promoters or hybrid promoters. Hybrid promoters, which combine elements of more than one promoter, are also known in the art, and are useful in the present invention.

In addition, the expression vector may comprise additional elements. For example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification. Furthermore, for integrating expression vectors, the expression vector contains at least one sequence homologous to the host cell genome, and preferably two homologous sequences which flank the expression construct. The integrating vector may be directed to a specific locus in the host cell by selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are well known in the art.

In addition, in a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

The HAP proteins of the present invention are produced by culturing a host cell transformed with an expression vector containing nucleic acid encoding a HAP protein, under the appropriate conditions to induce or cause expression of the HAP protein. The conditions appropriate for HAP protein expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. For example, the use of constitutive promoters in the expression vector will require optimizing the growth and proliferation of the host cell, while the use of an inducible promoter requires the appropriate growth conditions for induction. In addition, in some embodiments, the timing of the harvest is important. For example, the baculoviral systems used in insect cell expression are lytic viruses, and thus harvest time selection can be crucial for product yield.

Appropriate host cells include yeast, bacteria, archaeobacteria, fungi, and insect and animal cells, including mammalian cells. Of particular interest are *Drosophila melanogaster* cells, *Saccharomyces cerevisiae* and other yeasts, *E. coli*, *Bacillus subtilis*, SF9 cells, C129 cells, 293 cells, Neurospora, BHK, CHO, COS, and HeLa cells, immortalized mammalian myeloid and lymphoid cell lines.

In a preferred embodiment, HAP proteins are expressed in bacterial systems. Bacterial expression systems are well known in the art.

A suitable bacterial promoter is any nucleic acid sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of the coding sequence of HAP protein into mRNA. A bacterial promoter has a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region typically includes an RNA polymerase binding site and a transcription initiation site. Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose and maltose, and sequences derived from biosynthetic enzymes such as tryptophan. Promoters from bacteriophage may also be used and are known in the art. In addition, synthetic promoters and hybrid promoters

are also useful; for example, the *tac* promoter is a hybrid of the *trp* and *lac* promoter sequences. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription.

In addition to a functioning promoter sequence, an efficient ribosome binding site is desirable. In *E. coli*, the ribosome binding site is called the Shine-Delgarno (SD) sequence and includes an initiation codon and a sequence 3–9 nucleotides in length located 3–11 nucleotides upstream of the initiation codon.

The expression vector may also include a signal peptide sequence that provides for secretion of the HAP protein in bacteria. The signal sequence typically encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell, as is well known in the art. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria).

The bacterial expression vector may also include a selectable marker gene to allow for the selection of bacterial strains that have been transformed. Suitable selection genes include genes which render the bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin, neomycin and tetracycline. Selectable markers also include biosynthetic genes, such as those in the histidine, tryptophan and leucine biosynthetic pathways.

These components are assembled into expression vectors. Expression vectors for bacteria are well known in the art, and include vectors for *Bacillus subtilis*, *E. coli*, *Streptococcus cremoris*, and *Streptococcus lividans*, among others.

The bacterial expression vectors are transformed into bacterial host cells using techniques well known in the art, such as calcium chloride treatment, electroporation, and others.

In one embodiment, HAP proteins are produced in insect cells. Expression vectors for the transformation of insect cells, and in particular, baculovirus-based expression vectors, are well known in the art. Briefly, baculovirus is a very large DNA virus which produces its coat protein at very high levels. Due to the size of the baculoviral genome, exogenous genes must be placed in the viral genome by recombination. Accordingly, the components of the expression system include: a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the HAP protein; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the homologous recombination of the heterologous gene into the baculovirus genome); and appropriate insect host cells and growth media.

Mammalian expression systems are also known in the art and are used in one embodiment. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence for HAP protein into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, using a located 25–30 base pairs upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, typically located within 100 to 200 base pairs upstream of the TATA box. An upstream promoter element determines the rate at which transcription

is initiated and can act in either orientation. Of particular use as mammalian promoters are the promoters from mammalian viral genes, since the viral genes are often highly expressed and have a broad host range. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter, and herpes simplex virus promoter.

Typically, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-translational cleavage and polyadenylation. Examples of transcription terminator and polyadenylation signals include those derived from SV40.

The methods of introducing exogenous nucleic acid into mammalian hosts, as well as other hosts, is well known in the art, and will vary with the host cell used. Techniques include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide (s) in liposomes, and direct microinjection of the DNA into nuclei.

In a preferred embodiment, HAP protein is produced in yeast cells. Yeast expression systems are well known in the art, and include expression vectors for *Saccharomyces cerevisiae*, *Candida albicans* and *C. maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis* and *K. lactis*, *Pichia quillerimondii* and *P. pastoris*, *Schizosaccharomyces pombe*, and *Yarrowia lipolytica*. Preferred promoter sequences for expression in yeast include the inducible GAL1, 10 promoter, the promoters from alcohol dehydrogenase, enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase, hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, pyruvate kinase, and the acid phosphatase gene. Yeast selectable markers include ADE2, HIS4, LEU2, TRP1, and ALG7, which confers resistance to tunicamycin; the G418 resistance gene, which confers resistance to G418; and the CUP1 gene, which allows yeast to grow in the presence of copper ions.

A recombinant HAP protein may be expressed intracellularly or secreted. The HAP protein may also be made as a fusion protein, using techniques well known in the art. Thus, for example, if the desired epitope is small, the HAP protein may be fused to a carrier protein to form an immunogen. Alternatively, the HAP protein may be made as a fusion protein to increase expression.

Also included within the definition of HAP proteins of the present invention are amino acid sequence variants. These variants fall into one or more of three classes: substitutional, insertional or deletional variants. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the HAP protein, using cassette mutagenesis or other techniques well known in the art, to produce DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture as outlined above. However, variant HAP protein fragments having up to about 100–150 residues may be prepared by in vitro synthesis using established techniques. Amino acid sequence variants are characterized by the predetermined nature of the variation, a feature that sets them apart from naturally occurring allelic or interspecies variation of the HAP protein amino acid sequence. The variants typically exhibit the same qualitative biological activity as the naturally occurring analogue, although variants can also be selected which have modified characteristics as will be more fully outlined below.

While the site or region for introducing an amino acid sequence variation is predetermined, the mutation per se need not be predetermined. For example, in order to optimize the performance of a mutation at a given site, random mutagenesis may be conducted at the target codon or region and the expressed HAP protein variants screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example, M13 primer mutagenesis. Screening of the mutants is done using assays of HAP protein activities; for example, mutated HAP genes are placed in HAP deletion strains and tested for HAP activity, as disclosed herein. The creation of deletion strains, given a gene sequence, is known in the art. For example, nucleic acid encoding the variants may be expressed in a *Haemophilus influenzae* strain deficient in the HAP protein, and the adhesion and infectivity of the variant *Haemophilus influenzae* evaluated. Alternatively, the variant HAP protein may be expressed and its biological characteristics evaluated, for example its proteolytic activity.

Amino acid substitutions are typically of single residues; insertions usually will be on the order of from about 1 to 20 amino acids, although considerably larger insertions may be tolerated. Deletions range from about 1 to 30 residues, although in some cases deletions may be much larger, as for example when one of the domains of the HAP protein is deleted.

Substitutions, deletions, insertions or any combination thereof may be used to arrive at a final derivative. Generally these changes are done on a few amino acids to minimize the alteration of the molecule. However, larger changes may be tolerated in certain circumstances.

When small alterations in the characteristics of the HAP protein are desired, substitutions are generally made in accordance with the following chart:

CHART I

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	Met, Leu, Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those shown in Chart I. For example, substitutions may be made which more significantly affect: the structure of the polypeptide backbone in the area of the alteration, for example the alpha-helical or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the polypeptide's properties are those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic

residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g. lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g. glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g. phenylalanine, is substituted for (or by) one not having a side chain, e.g. glycine.

The variants typically exhibit the same qualitative biological activity and will elicit the same immune response as the naturally-occurring analogue, although variants also are selected to modify the characteristics of the polypeptide as needed. Alternatively, the variant may be designed such that the biological activity of the HAP protein is altered. For example, the proteolytic activity of the larger 110 kD domain of the HAP protein may be altered, through the substitution of the amino acids of the active site. The putative catalytic domain of this protein is GDSGSPMF (SEQ ID NO:7), with the first serine corresponding to the active site serine characteristic of serine type proteases. The residues of the active site may be individually or simultaneously altered to decrease or eliminate proteolytic activity. This may be done to decrease the toxicity or side effects of the vaccine. Similarly, the cleavage site between the 45 kD domain and the 100 kD domain may be altered, for example to eliminate proteolytic processing to form the two domains. Putatively this site is at residue 960.

In a preferred embodiment, the HAP protein is purified or isolated after expression. HAP proteins may be isolated or purified in a variety of ways known to those skilled in the art depending on what other components are present in the sample. Standard purification methods include electrophoretic, molecular, immunological and chromatographic techniques, including ion exchange, hydrophobic, affinity, and reverse-phase HPLC chromatography, and chromatofocusing. For example, the HAP protein may be purified using a standard anti-HAP antibody column. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. For general guidance in suitable purification techniques, see Scopes, R., Protein Purification, Springer-Verlag, NY (1982). The degree of purification necessary will vary depending on the use of the HAP protein. In some instances no purification will be necessary.

Once expressed and purified if necessary, the HAP proteins are useful in a number of applications.

For example, the HAP proteins can be coupled, using standard technology, to affinity chromatography columns. These columns may then be used to purify antibodies from samples obtained from animals or patients exposed to the *Haemophilus influenzae* organism. The purified antibodies may then be used as outlined below.

Additionally, the HAP proteins are useful to make antibodies to HAP proteins. These antibodies find use in a number of applications. In a preferred embodiment, the antibodies are used to diagnose the presence of an *Haemophilus influenzae* infection in a sample or patient. This will be done using techniques well known in the art; for example, samples such as blood or tissue samples may be obtained from a patient and tested for reactivity with the antibodies, for example using standard techniques such as ELISA. In a preferred embodiment, monoclonal antibodies are generated to the HAP protein, using techniques well known in the art. As outlined above, the antibodies may be generated to the full length HAP protein, or a portion of the HAP protein.

Antibodies generated to HAP proteins may also be used in passive immunization treatments, as is known in the art.

Antibodies generated to unique sequences of HAP proteins may also be used to screen expression libraries from other organisms to find, and subsequently clone, HAP nucleic acids from other organisms.

In one embodiment, the antibodies may be directly or indirectly labelled. By "labelled" herein is meant a compound that has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) immune labels, which may be antibodies or antigens; and c) colored or fluorescent dyes. The labels may be incorporated into the compound at any position. Thus, for example, the HAP protein antibody may be labelled for detection, or a secondary antibody to the HAP protein antibody may be created and labelled.

In one embodiment, the antibodies generated to the HAP proteins of the present invention are used to purify or separate HAP proteins or the *Haemophilus influenzae* organism from a sample. Thus for example, antibodies generated to HAP proteins which will bind to the *Haemophilus influenzae* organism may be coupled, using standard technology, to affinity chromatography columns. These columns can be used to pull out the *Haemophilus* organism from environmental or tissue samples. Alternatively, antibodies generated to the soluble 110 kD portion of the full-length portion of the protein shown in FIG. 7 may be used to purify the 110 kD protein from samples.

In a preferred embodiment, the HAP proteins of the present invention are used as vaccines for the prophylactic or therapeutic treatment of a *Haemophilus influenzae* infection in a patient. By "vaccine" herein is meant an antigen or compound which elicits an immune response in an animal or patient. The vaccine may be administered prophylactically, for example to a patient never previously exposed to the antigen, such that subsequent infection by the *Haemophilus influenzae* organism is prevented. Alternatively, the vaccine may be administered therapeutically to a patient previously exposed or infected by the *Haemophilus influenzae* organism. While infection cannot be prevented, in this case an immune response is generated which allows the patient's immune system to more effectively combat the infection. Thus, for example, there may be a decrease or lessening of the symptoms associated with infection.

A "patient" for the purposes of the present invention includes both humans and other animals and organisms. Thus the methods are applicable to both human therapy and veterinary applications.

The administration of the HAP protein as a vaccine is done in a variety of ways. Generally, the HAP proteins can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby therapeutically effective amounts of the HAP protein are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation are well known in the art. Such compositions will contain an effective amount of the HAP protein together with a suitable amount of vehicle in order to prepare pharmaceutically acceptable compositions for effective administration to the host. The composition may include salts, buffers, carrier proteins such as serum albumin, targeting molecules to localize the HAP protein at the appropriate site or tissue within the organism, and other molecules. The composition may include adjuvants as well.

In one embodiment, the vaccine is administered as a single dose; that is, one dose is adequate to induce a

sufficient immune response to prophylactically or therapeutically treat a *Haemophilus influenzae* infection. In alternate embodiments, the vaccine is administered as several doses over a period of time, as a primary vaccination and "booster" vaccinations.

By "therapeutically effective amounts" herein is meant an amount of the HAP protein which is sufficient to induce an immune response. This amount may be different depending on whether prophylactic or therapeutic treatment is desired. Generally, this ranges from about 0.001 mg to about 1 gm, with a preferred range of about 0.05 to about, and the preferred dose being _____. These amounts may be adjusted if adjuvants are used.

The following examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes.

EXAMPLES

Example 1

Cloning of the HAP Protein

Bacterial strains, plasmids, and phage. *H. influenzae* strain N187 is a clinical isolate that was originally cultivated from the middle ear fluid of a child with acute otitis media. This strain was classified as nontypable based on the absence of agglutination with typing antisera for *H. Influenzae* types a-f (Burroughs Wellcome) and the failure to hybridize with pU038, a plasmid that contains the entire cap b locus (Kroll and Moxon, 1988, J. Bacteriol. 170:859-864).

H. influenzae strain DB117 is a red mutant of Rd, a capsule-deficient serotype d strain that has been in the laboratory for over 40 years (Alexander and Leidy, 1951, J. Exp. Med. 83:345-359); DB117 was obtained from G. Barcak (University of Maryland, Baltimore, Md.) (Sellow et al., 1968). DB117 is deficient for in vitro adherence and invasion, as assayed below.

H. influenzae strain 12 is the nontypable strain from which the genes encoding the HMW1 and HMW2 proteins were cloned (Barenkamp and Leininger, 1992, Infect. Immun. 60:1302-1313); HMW1 and HMW2 are the prototypic members of a family of nontypable *Haemophilus* antigenically-related high-molecular-weight adhesive proteins (St. Geme et al., 1993).

E. coli HB101, which is nonadherent and noninvasive, has been previously described (Sambrook et al., 1989, Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). *E. coli* DH5 α was obtained from Bethesda Research Laboratories. *E. coli* MC1061 was obtained from H. Kimsey (Tufts University, Boston, Mass.). *E. coli* XL-1 Blue and the plasmid pBluescript KS- were obtained from Stratagene. Plasmid pT7-7 and phage mGP1-2 were provided by S. Tabor (Harvard Medical School, Boston, Mass.) (Tabor and Richardson, 1985, Proc. Natl. Acad. Sci. USA. 82:1074-1078). The *E. coli*-*Haemophilus* shuttle vector pGJB103 (Tomb et al., 1989, Rd. J. Bacteriol. 171:3796-3802) and phage λ 1105 (Way et al., 1984, Gene. 32:369-379) were provided by G. Barcak (University of Maryland, Baltimore, Md.). Plasmid pVD116 harbors the IgA1 protease gene from *H. influenzae* strain Rd (Kooimey and Falkow, 1984, Infect. Immun. 43:101-107) and was obtained from M. Kooimey (University of Michigan, Ann Arbor, Mich.).

Growth conditions. *H. influenzae* strains were grown as described (Anderson et al., 1972, J. Clin. Invest. 51:31-38). They were stored at -80° C. in brain heart infusion broth with 25% glycerol. *E. coli* strains were grown on LB agar or in LB broth. They were stored at -80° C. in LB broth with 50% glycerol.

For *H. influenzae*, tetracycline was used in a concentration of 5 μ g/ml and kanamycin was used in a concentration of 25 μ g/ml. For *E. coli*, antibiotics were used in the following concentrations: tetracycline, 12.5 μ g/ml; kanamycin, 50 μ g/ml; ampicillin, 100 μ g/ml.

Recombinant DNA methods. DNA ligations, restriction endonuclease digestions, and gel electrophoresis were performed according to standard techniques (Sambrook et al., 1989, supra). Plasmids were introduced into *E. coli* strains by either chemical transformation or electroporation, as described (Sambrook et al., 1989, supra; Dower et al., 1988, Nucleic Acids Res. 16:617-6145). In *H. influenzae* transformation was performed using the MIV method of Herriott et al. (1970, J. Bacteriol. 101:517-524), and electroporation was carried out using the protocol developed for *E. coli* (Dower et al., 1988, supra).

Construction of genomic library from *H. influenzae* strain N187. High-molecular-weight chromosomal DNA was prepared from 3 ml of an overnight broth culture of *H. influenzae* N187 as previously described (Mekalanos, 1983, Cell. 35:253-263). Following partial digestion with Sau3AI, 8 to 12 kb fragments were eluted into DEAE paper (Schleicher & Schuell, Keene, H. H.) and then ligated to BglIII-digested calf intestine phosphatase-treated pGJB103. The ligation mixture was electroporated into *H. influenzae* DB117, and transformants were selected on media containing tetracycline.

Transposon Mutagenesis.

Mutagenesis of plasmid DNA was performed using the mini-Tn10 kan element described by Way et al. (1984, supra). Initially, the appropriate plasmid was introduced into *E. coli* MC1061. The resulting strain was infected with λ 1105, which carries the mini-Tn10 kan transposon. Transductants were grown overnight in the presence of kanamycin and an antibiotic to select for the plasmid, and plasmid DNA was isolated using the alkaline lysis method. In order to recover plasmids containing a transposon insertion, plasmid DNA was electroporated into *E. coli* DH5 α , plating on media containing kanamycin and the appropriate second antibiotic.

In order to establish more precisely the region of pN187 involved in promoting interaction with host cells, initially this plasmid was subjected to restriction endonuclease analysis. Subsequently, several subclones were constructed in the vector pGJB103 and were reintroduced into *H. influenzae* strain DB117. The resulting strains were then examined for adherence and invasion. As summarized in FIG. 4, subclones containing either a 3.9-kb PstI-BglIII fragment (pJS105) or the adjoining 4.2-kb BglIII fragment (pJS102) failed to confer the capacity to associate with Chang cells. In contrast, a subclone containing an insert that included portions of both of these fragments (pJS106) did promote interaction with epithelial monolayers. Transposon mutagenesis performed on pH 187 confirmed that the flanking portions of the insert in this plasmid were not required for the adherent/invasive phenotype. On the other hand, a transposon insertion located adjacent to the BglIII site in pJS106 eliminated adherence and invasion. An insertion between the second EcoRI and PstI sites in this plasmid had a similar effect (FIG. 4).

Examination of Plasmid-encoded Proteins.

In order to examine plasmid encoded proteins, relevant DNA was ligated into the bacteriophage T7 expression vector pT7-7, and the resulting construct was transformed into *E. coli* XL-1 Blue. Plasmid pT7-7 contains the T7 phage ϕ 10 promoter and ribosomal binding site upstream of a multiple cloning site (Tabor and Richardson, 1985, supra). The T7 promoter was induced by infection with the recombinant M13 phage mGP1-2 and addition of isopropyl- β -D-thiogalactopyranoside (final concentration, 1 mM). Phage mGP1-2 contains the gene encoding T7 RNA polymerase, which activates the ϕ 10 promoter in pT7-7 (Tabor and Richardson, 1985, supra).

Like DB117(pN187), strain DB117 carrying pJS106 expressed new outer membrane proteins 160-kD and 45-kD in size (FIG. 3, lane 3). In order to examine whether the 6.5-kb insert in pJS106 actually encodes these proteins, this fragment of DNA was ligated into the bacteriophage T7 expression vector pT7-7. The resulting plasmid containing the insert in the same orientation as in pN187 was designated pJS104, and the plasmid with the insert in the opposite orientation was designated pJS103. Both pJS104, and p7S103 were introduced into *E. coli* XL-1 Blue, producing XL-1 Blue(pJS104) and XL-1 Blue(pJS103), respectively. As a negative control, pT7-7 was also transformed into XL-1 Blue. The T7 promoter was induced in these three strains by infection with the recombinant M13 phage mGP1-2 and addition of isopropyl- β -D-thiogalactopyranoside (final concentration, 1 mM), and induced proteins were detected using [³⁵S] methionine. As shown in FIG. 5, induction of XL-1 Blue(pJS104) resulted in expression of a 160-kD protein and several smaller proteins which presumably represent degradation products. In contrast, when XL-1 Blue (pJS103) and XL-1 Blue(pT7-7) were induced, there was no expression of these proteins. There was no 45-kD protein induced in any of the three strains. This experiment suggested that the 6.5-kb insert present in pJS106 contains the structural gene for the 160-kD outer membrane protein identified in DB117(pJS106). On the other hand, this analysis failed to establish the origin of the 45-kD membrane protein expressed by DB117(pJS106).

Adherence and Invasion Assays.

Adherence and invasion assays were performed with Chang epithelial cells [Wong-Kilbourne derivative, clone 1-5c-4 (human conjunctiva)], which were seeded into wells of 24-well tissue culture plates as previously described (St. Geme and Falkow, 1990). Adherence was measured after incubating bacteria with epithelial monolayers for 30 minutes as described (St. Geme et al., 1993). Invasion assays were carried out according to our original protocol and involved incubating bacteria with epithelial cells for four hours followed by treatment with gentamicin for two hours (100 μ g/ml) (St. Geme and Falkow, 1990).

Nucleotide Sequence Determination and Analysis.

Nucleotide sequence was determined using a Sequenase kit and double stranded plasmid template. DNA fragments were subcloned into pBluescript KS- and sequenced along both strands by primer walking. DNA sequence analysis was performed using the Genetics Computer Group (GCG) software package from the University of Wisconsin (Devereux et al., 1984). Sequence similarity searches were carried out using the BLAST program of the National Center for Biotechnology Information (Altschul et al., 1990, J. Mol. Biol. 215:403-410). The DNA sequence described here will be deposited in the EMBL/GenBank/DDBJ Nucleotide Sequence Data Libraries.

Based on our subcloning results, we reasoned that the central BglII site in pN187 was positioned within an open reading frame. Examination of a series of mini-Tn10 kan

mutants supported this conclusion (FIG. 4). Consequently, we sequenced DHA on either side of this BglII site and identified a 4182 bp gene, which we have designated hap for Haemophilus adherence and penetration (FIG. 6). This gene encodes a 1394 amino acid polypeptide, which we have called Hap, with a calculated molecular mass of 155.4-kD, in good agreement with the molecular mass of the larger of the two novel outer membrane proteins expressed by DB117 (pN187) and the protein expressed after induction of XL-1 Blue/pJS104. The hap gene has a G+C content of 39.1%, similar to the published estimate of 38.7% for the whole genome (Kilian, 1976, J. Gen. Microbiol. 93:9-62). Putative -10 and -35 promoter sequences are present upstream of the initiation codon. A consensus ribosomal binding site is lacking. A sequence similar to a rho-independent transcription terminator is present beginning 39 nucleotides beyond the stop codon and contains interrupted inverted repeats with the potential for forming a hairpin structure containing a loop of three bases and a stem of eight bases. Similar to the situation with typical *E. coli* terminators, this structure is followed by a stretch rich in T residues. Analysis of the predicted amino acid sequence suggested the presence of a 25 amino acid signal peptide at the amino terminus. This region has characteristics typical of procaryotic signal peptides, with three positive H-terminal charges, a central hydrophobic region, and alanine residues at positions 23 and 25 (-3 and -1 relative to the putative cleavage site) (von Heijne, 1984, J. Mol. Biol. 173:243-251).

Comparison of the deduced amino acid sequence of Hap with other proteins. A protein sequence similarity search was performed with the predicted amino acid sequence using the BLAST network service of the National Center for Biotechnology Information (Altschul et al., 1990, supra). This search revealed homology with the IgA1 proteases of *H. influenzae* and *Neisseria gonorrhoeae*. Alignment of the derived amino acid sequences for the hap gene product and the IgA1 proteases from four different *H. influenzae* strains revealed homology across the extent of the proteins (FIG. 7), with several stretches showing 55-60% identity and 70-80% similarity. Similar levels of homology were noted between the hap product and the IgA1 protease from *N. Gonorrhoeae* strain MS11. This homology includes the region identified as the catalytic site of the IgA1 proteases, which is comprised of the sequence GDSGSPLF (SEQ ID NO:8), where 2 is the active site serine characteristic of serine proteases (Brenner, 1988, Nature (London). 334:528-530; Poulsen et al., 1992, J. Bacteriol. 174:2913-2921). In the case of Hap, the corresponding sequence is GDSGSMPF (SEQ ID NO:7). The hap product also contains two cysteines corresponding to the cysteines proposed to be important in forming the catalytic domain of the IgA proteases (Pohiner et al., 1987, supra). Overall there is 30-35% identity and 51-55% similarity between the hap gene product and the *H. influenzae* and *N. gonorrhoeae* IgA proteases.

The deduced amino acid sequence encoded by hap was also found to contain significant homology to Tsh, a hemagglutinin expressed by an avian *E. coli* strain (Provence and Curtiss, 1994, supra). This homology extends throughout both proteins but is greatest in the H-terminal half of each. Overall the two proteins are 30.5% identical and 51.6% similar. Tsh is also synthesized as a preprotein and is secreted as a smaller form; like the IgA1 proteases and perhaps Hap, a carboxy terminal peptide remains associated with the outer membrane (D. Provence, personal communication). While this protein is presumed to have proteolytic activity, its substrate has not yet been determined. Interestingly, Tsh was first identified on the basis of its capacity to promote agglutination of erythrocytes. Thus Hap and Tsh are possibly the first members of a novel class of adhesive proteins that are processed analogously to the IgA1 proteases.

Homology was also noted with pertactin, a 69-kD outer membrane protein expressed by *B. pertussis* (Charles et al., 1989, Proc. Natl. Acad. Sci. USA. 86:3554–3558). The middle portions of these two molecules are 39% identical and nearly 60% similar. This protein contains the amino acid triplet arginine-glycine-aspartic acid (RGD) and has been shown to promote attachment to cultured mammalian cells via this sequence (Leininger et al., 1991, Proc. Natl. Acad. Sci. USA. 88:345–349). Although Bordetella species are not generally considered intracellular parasites, work by Ewanowich and coworkers indicates that these respiratory pathogens are capable of in vitro entry into human epithelial cells (Ewanowich et al., 1989, Infect. Immun. 57:2698–2704; Ewanowich et al., 1989, Infect. Immun. 57:1240–1247). Recently Leininger et al. reported that pre-incubation of epithelial monolayers with an RGD-containing peptide derived from the pertactin sequence specifically inhibited *B. pertussis* entry (Leininger et al., 1992, Infect. Immun. 60:2380–2385). In addition, these investigators found that coating of *Staphylococcus aureus* with purified pertactin resulted in more efficient *S. aureus* entry; the RGD-containing peptide from pertactin inhibited this pertactin-enhanced entry by 75%. Although the hap product lacks an RGD motif, it is possible that Hap and pertactin serve similar biologic functions for *H. influenzae* and Bordetella species, respectively.

Additional analysis revealed significant homology (34 to 52% identity, 42 to 70% similarity) with six regions of HpmA, a calcium-independent hemolysin expressed by *Proteus mirabilis* (Uphoff and Welch, 1990, supra).

The hap Locus is Distinct from the *H. influenzae* IgA1 Protease Gene.

Given the degree of similarity between the hap gene product and *H. influenzae* IgA1 protease, we wondered whether we had isolated the IgA1 protease gene of strain N187. To examine this possibility, we performed IgA1 protease activity assays. Among *H. influenzae* strains, two enzymatically distinct types of IgA1 protease have been found (Mulks et al., 1982, J. Infect. Dis. 146:266–274). Type 1 enzymes cleave the Pro-Ser peptide bond between residues 231 and 232 in the hinge region of human IgA1 heavy chain and generate fragments of roughly 28-kD and 31-kD; type 2 enzymes cleave the Pro-Thr bond between residues 235 and 236 in the hinge region and generate 26.5-kD and 32.5-kD fragments. Previous studies of the parent strain from which DB117 was derived have demonstrated that this strain produces a type 1 IgA1 protease (Koomey and Falkow, 1984, supra). As shown in FIG. 8, comparison of the proteolytic activities of strain DB117 and strain N187 suggested that N187 produces a type 2 IgA1 protease. We reasoned that DB117(pN187) might generate a total of four fragments from IgA1 protease, consistent with two distinct cleavage specificities. Examination of DB117(pH187) revealed instead that this transformant produces the same two fragments of the IgA1 heavy chain as does DB117, arguing that this strain produces only a type 1 enzyme.

In an effort to obtain additional evidence against the possibility that plasmid pH187 contains the N187 IgA1 protease gene, we performed a series of Southern blots. As shown in FIG. 9, when genomic DNA from strain N187 was digested with EcoRI, BglII, or BanHI and then probed with the hap gene, one set of hybridizing fragments was detected. Probing of the same DNA with the iga gene from *H. influenzae* strain Rd resulted in a different set of hybridizing bands. Moreover, the iga gene failed to hybridize with a purified 4.8-kb fragment that contained the intact hap gene.

The Recombinant Plasmid Associated with Adherence and Invasion Encodes a Secreted Protein.

The striking homology between the hap gene product and the Haemophilus and Neisseria IgA1 proteases suggested

the possibility that these proteins might be processed in a similar manner. The IgA1 proteases are synthesized as preproteins with three functional domains: the N-terminal signal peptide, the protease, and a C-terminal helper domain, which is postulated to form a pore in the outer membrane for secretion of the protease (Poulsen et al., 1989, supra; Pohlner et al., 1987, supra). The C-terminal peptide remains associated with the outer membrane following an autoproteolytic cleavage event that results in release of the mature enzyme.

Consistent with the possibility that the hap gene product follows a similar fate, we found that DB117(pN187) produced a secreted protein approximately 110-kD in size that was absent from DB117(pGJB103) (FIG. 10). This protein was also produced by DB117(pJS106), but not by DB117(pJ5102) or DB117(pJS105). Furthermore, the two mutants with transposon insertions within the hap coding region were deficient in this protein. In order to determine the relationship between hap and the secreted protein, this protein was transferred to a PVDF membrane and N-terminal amino acid sequencing was performed. Excessive background on the first cycle precluded identification of the first amino acid residue of the free amino terminus. The sequence of the subsequent seven residues was found to be HTYFGID (SEQ ID NO:9), which corresponds to amino acids 27 through 33 of the hap product.

The introduction of hap into laboratory strains of *E. coli* strains was unable to endow these organisms with the capacity for adherence or invasion. In considering these results, it is noteworthy that the *E. coli* transformants failed to express either the 160-kD or the 45-kD outer membrane protein. Accordingly, they also failed to express the 110-kD secreted protein. The explanation for this lack of expression is unclear. One possibility is that the *H. influenzae* promoter or ribosomal binding site was poorly recognized in *E. coli*. Indeed the putative -35 sequence upstream of the hap initiation codon is fairly divergent from the $\sigma 70$ consensus sequence, and the ribosomal binding site is unrecognizable. Alternatively, an accessory gene may be required for proper export of the Hap protein, although the striking homology with the IgA proteases, which are normally expressed and secreted in *E. coli*, argues against this hypothesis.

In considering the possibility that the hap gene product promotes adherence and invasion by directly binding to a host cell surface structure, it seems curious that the mature protein is secreted from the organism. However, there are examples of other adherence factors that are also secreted. Filamentous hemagglutinin is a 220-kD protein expressed by *B. pertussis* that mediates in vitro adherence and facilitates natural colonization (Relman et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:2637–2641; Kimura et al., 1990, Infect. Immun. 58:7–16). This protein remains surface-associated to some extent but is also released from the cell. The process of Filamentous hemagglutinin secretion involves an accessory protein designated FhaC, which appears to be localized to the outer membrane (Willems et al., 1994, Molec. Microbiol. 11:337–347). Similarly, the Ipa proteins implicated in Shigella invasion are also secreted. Secretion of these proteins requires the products of multiple genes within the mxi and spa loci (Allaoui et al., 1993, Molec. Microbiol. 7:59–68; Andrews et al., 1991, Infect. Immun. 59:1997–2005; Venkatsan et al., 1992, J. Bacteriol. 174:1990–2001).

It is conceivable that secretion is simply a consequence of the mechanism for export of the hap gene product to the surface of the organism. However, it is noteworthy that the secreted protein contains a serine-type protease catalytic domain and shows homology with the *P. mirabilis* hemolysin. These findings suggest that the mature Hap protein may possess proteolytic activity and raise the possibility that

Hap promotes interaction with the host cell at a distance by modifying the host cell surface. Alternatively, Hap may modify the bacterial surface in order to facilitate interaction with a host cell receptor. It is possible that hap encodes a molecule with dual functions, serving as both adhesin and protease.

Analysis of Outer Membrane and Secreted Proteins.

Outer membrane proteins were isolated on the basis of sarcosyl insolubility according to the method of Carlone et al. (1986, *J. clin. Microbiol.* 24:330-332). Secreted proteins were isolated by centrifuging bacterial cultures at 16,000 g for 10 minutes, recovering the supernatant, and precipitating with trichloroacetic acid in a final concentration of 10%. SDS-polyacrylamide gel electrophoresis was performed as previously described (Laemmli, 1970, *Nature (London)*. 227:680-685).

To identify proteins that might be involved in the interaction with the host cell surface, outer membrane protein profiles for DB117(pN187) and DB117(pGJB103) were compared. As shown in FIG. 3, DB117(pN187) expressed two new outer membrane proteins: a high-molecular-weight protein approximately 160-kD in size and a 45-kD protein. *E. coli* HB101 harboring pN187 failed to express these proteins, suggesting an explanation for the observation that HB101(pN187) is incapable of adherence or invasion.

Previous studies have demonstrated that a family of antigenically-related high-molecular-weight proteins with similarity to filamentous hemagglutinin of *Bordetella pertussis* mediate attachment by nontypable *H. influenzae* to cultured epithelial cells (St. Geme et al., 1993). To explore the possibility that the gene encoding the strain H187 member of this family was cloned, whole cell lysates of N187, DB117(pN187), and DB117 (pGJB103) were examined by Western immunoblot. Our control strain for this experiment was *H. influenzae* strain 12. Using a polyclonal antiserum directed against HMW1 and HMW2, the prototypic proteins in this family, we identified a 140-kD protein in strain H187 (not shown). In contrast, this antiserum failed to react with either DB117(pN187) or DB117(pGJB103) (not shown), indicating that pN187 has no relationship to HMW protein expression.

Determination of amino terminal sequence. Secreted proteins were precipitated with trichloroacetic acid, separated on a 10% SDS-polyacrylamide gel, and electrotransferred to a polyvinylidene difluoride (PVDF) membrane (Matsudaira, 1987, *J. Biol. Chem.* 262:10035-10038). Following staining with Coomassie Brilliant Blue R-250, the 110-kD protein was cut from the PVDF membrane and submitted to the Protein Chemistry Laboratory at Washington University School of Medicine for amino terminal sequence determination. Sequence analysis was performed by automated Edman degradation using an Applied Biosystems Model 470A protein sequencer.

Examination of IgA1 protease activity. In order to assess IgA1 protease activity, bacteria were inoculated into broth and grown aerobically overnight. Samples were then centrifuged in a microphage for two minutes, and supernatants were collected. A 10 μ l volume of supernatant was mixed with 16 μ l of 0.5 μ g/ml human IgA1 (Calbiochem), and chloramphenicol was added to a final concentration of 2 μ g/ml. After overnight incubation at 37° C., reaction mixtures were electrophoresed on a 10% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane, and probed with goat anti-human IgA1 heavy chain conjugated to alkaline phosphatase (Kirkegaard & Perry). The membrane was developed by immersion in phosphatase substrate solution (5-bromo-4-chloro-3-indolylphosphate toluidinium-nitro blue tetrazolium substrate system; Kirkegaard & Perry).

Immunoblot analysis. Immunoblot analysis of bacterial whole cell lysates was carried out as described (St. Geme et al., 1991).

Southern hybridization. Southern blotting was performed using high stringency conditions as previously described (St. Geme and Falkow, 1991).

Microscopy.

i. Light microscopy. Samples of epithelial cells with associated bacteria were stained with Giemsa stain and examined by light microscopy as described (St. Geme and Falkow, 1990).

ii. Transmission electron microscopy. For transmission electron microscopy, bacteria were incubated with epithelial cell monolayers for four hours and were then rinsed four times with PBS, fixed with 2% glutaraldehyde/1% osmium tetroxide in 0.1 M sodium phosphate buffer pH 6.4 for two hours on ice, and stained with 0.25% aqueous uranyl acetate overnight. Samples were then dehydrated in graded ethanol solutions and embedded in polybed. Ultrathin sections (0.4 μ m) were examined in a Phillips 201c electron microscope.

As shown in FIG. 2, DB117(pN187) incubated with monolayers for four hours demonstrated intimate interaction with the epithelial cell surface and was occasionally found to be intracellular. In a given thin section, invaded cells generally contained one or two intracellular organisms. Of note, intracellular bacteria were more common in sections prepared with strain N187, an observation consistent with results using the gentamicin assay. In contrast, examination of samples prepared with strain DB117 carrying cloning vector alone (pGJB103) failed to reveal internalized bacteria (not shown).

Having described the preferred embodiments of the present invention it will appear to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments, and that such modifications are intended to be within the scope of the present invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 9

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4319 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

-continued

(D) TOPOLOGY: both

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 60..4241

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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20 25 30	
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35 40 45	
GTT GGG GCT CAA AAT ATT AAG GTT TAT AAC AAA CAA GGG CAA TTA GTT	251
Val Gly Ala Gln Asn Ile Lys Val Tyr Asn Lys Gln Gly Gln Leu Val	
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65 70 75 80	
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85 90 95	
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Ala His Asn Val Gly Tyr Thr Asp Val Asp Phe Gly Ala Glu Gly Asn	
100 105 110	
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Asn Pro Asp Gln His Arg Phe Thr Tyr Lys Ile Val Lys Arg Asn Asn	
115 120 125	
TAC AAA AAA GAT AAT TTA CAT CCT TAT GAG GAC GAT TAC CAT AAT CCA	491
Tyr Lys Lys Asp Asn Leu His Pro Tyr Glu Asp Asp Tyr His Asn Pro	
130 135 140	
CGA TTA CAT AAA TTC GTT ACA GAA GCG GCT CCA ATT GAT ATG ACT TCG	539
Arg Leu His Lys Phe Val Thr Glu Ala Ala Pro Ile Asp Met Thr Ser	
145 150 155 160	
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165 170 175	
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180 185 190	
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195 200 205	
CAC AAT CAG CGT GGA GCA GGT AAT GGA TAT TCG TAT TTG GGA GGC GAT	731
His Asn Gln Arg Gly Ala Gly Asn Gly Tyr Ser Tyr Leu Gly Gly Asp	
210 215 220	
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Val Arg Lys Ala Gly Glu Tyr Gly Pro Leu Pro Ile Ala Gly Ser Lys	
225 230 235 240	
GGG GAC AGT GGT TCT CCG ATG TTT ATT TAT GAT GCT GAA AAA CAA AAA	827
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245 250 255	
TGG TTA ATT AAT GGG ATA TTA CGG GAA GGC AAC CCT TTT GAA GGC AAA	875
Trp Leu Ile Asn Gly Ile Leu Arg Glu Gly Asn Pro Phe Glu Gly Lys	
260 265 270	
GAA AAT GGG TTT CAA TTG GTT CGC AAA TCT TAT TTT GAT GAA ATT TTC	923

-continued

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Glu	Arg	Asp	Leu	His	Thr	Ser	Leu	Tyr	Thr	Arg	Ala	Gly	Asn	Gly	Val		
	290					295				300							
TAC	ACA	ATT	AGT	GGA	AAT	GAT	AAT	GGT	CAG	GGG	TCT	ATA	ACT	CAG	AAA	1019	
Tyr	Thr	Ile	Ser	Gly	Asn	Asp	Asn	Gly	Gln	Gly	Ser	Ile	Thr	Gln	Lys		
	305			310					315						320		
TCA	GGA	ATA	CCA	TCA	GAA	ATT	AAA	ATT	ACG	TTA	GCA	AAT	ATG	AGT	TTA	1067	
Ser	Gly	Ile	Pro	Ser	Glu	Ile	Lys	Ile	Thr	Leu	Ala	Asn	Met	Ser	Leu		
			325						330						335		
CCT	TTG	AAA	GAG	AAG	GAT	AAA	GTT	CAT	AAT	CCT	AGA	TAT	GAC	GGA	CCT	1115	
Pro	Leu	Lys	Glu	Lys	Asp	Lys	Val	His	Asn	Pro	Arg	Tyr	Asp	Gly	Pro		
		340					345						350				
AAT	ATT	TAT	TCT	CCA	CGT	TTA	AAC	AAT	GGA	GAA	ACG	CTA	TAT	TTT	ATG	1163	
Asn	Ile	Tyr	Ser	Pro	Arg	Leu	Asn	Asn	Gly	Glu	Thr	Leu	Tyr	Phe	Met		
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GAT	CAA	AAA	CAA	GGA	TCA	TTA	ATC	TTC	GCA	TCT	GAC	ATT	AAC	CAA	GGG	1211	
Asp	Gln	Lys	Gln	Gly	Ser	Leu	Ile	Phe	Ala	Ser	Asp	Ile	Asn	Gln	Gly		
	370					375					380						
GCG	GGT	GGT	CTT	TAT	TTT	GAG	GGT	AAT	TTT	ACA	GTA	TCT	CCA	AAT	TCT	1259	
Ala	Gly	Gly	Leu	Tyr	Phe	Glu	Gly	Asn	Phe	Thr	Val	Ser	Pro	Asn	Ser		
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AAC	CAA	ACT	TGG	CAA	GGA	GCT	GGC	ATA	CAT	GTA	AGT	GAA	AAT	AGC	ACC	1307	
Asn	Gln	Thr	Trp	Gln	Gly	Ala	Gly	Ile	His	Val	Ser	Glu	Asn	Ser	Thr		
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GTT	ACT	TGG	AAA	GTA	AAT	GGC	GTG	GAA	CAT	GAT	CGA	CTT	TCT	AAA	ATT	1355	
Val	Thr	Trp	Lys	Val	Asn	Gly	Val	Glu	His	Asp	Arg	Leu	Ser	Lys	Ile		
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GGT	AAA	GGA	ACA	TTG	CAC	GTT	CAA	GCC	AAA	GGG	GAA	AAT	AAA	GGT	TCG	1403	
Gly	Lys	Gly	Thr	Leu	His	Val	Gln	Ala	Lys	Gly	Glu	Asn	Lys	Gly	Ser		
		435					440								445		
ATC	AGC	GTA	GGC	GAT	GGT	AAA	GTC	ATT	TTG	GAG	CAG	CAG	GCA	GAC	GAT	1451	
Ile	Ser	Val	Gly	Asp	Gly	Lys	Val	Ile	Leu	Glu	Gln	Gln	Ala	Asp	Asp		
		450				455						460					
CAA	GGC	AAC	AAA	CAA	GCC	TTT	AGT	GAA	ATT	GGC	TTG	GTT	AGC	GGC	AGA	1499	
Gln	Gly	Asn	Lys	Gln	Ala	Phe	Ser	Glu	Ile	Gly	Leu	Val	Ser	Gly	Arg		
	465				470					475					480		
GGG	ACT	GTT	CAA	TTA	AAC	GAT	GAT	AAA	CAA	TTT	GAT	ACC	GAT	AAA	TTT	1547	
Gly	Thr	Val	Gln	Leu	Asn	Asp	Asp	Lys	Gln	Phe	Asp	Thr	Asp	Lys	Phe		
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TAT	TTC	GGC	TTT	CGT	GGT	GGT	CGC	TTA	GAT	CTT	AAC	GGG	CAT	TCA	TTA	1595	
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			500					505							510		
ACC	TTT	AAA	CGT	ATC	CAA	AAT	ACG	GAC	GAG	GGG	GCA	ATG	ATT	GTG	AAC	1643	
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-continued

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CAT TTA AAT AAA CGT TGG TCA GAA ATG GAA GGT ATA CCA CAA GGC GAA	1979
His Leu Asn Lys Arg Trp Ser Glu Met Glu Gly Ile Pro Gln Gly Glu	
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TTC CAA ATT AAA GGC GGA AGT GCG GTG GTT TCT CGC AAT GTT TCT TCA	2075
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725 730 735	
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740 745 750	
ACT TTA ACA AAT CAC AGC CAA TTT ACA TTA AGC AAC AAT GCC ACC CAA	2363
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755 760 765	
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Ile Gly Asn Ile Arg Leu Ser Asp Asn Ser Thr Ala Thr Val Asp Asn	
770 775 780	
GCA AAC TTG AAC GGT AAT GTG CAT TTA ACG GAT TCA GCT CAA TTT TCT	2459
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Leu Lys Asn Ser His Phe Ser His Gln Ile Gln Gly Asp Lys Gly Thr	
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ACA GTG ACG TTG GAA AAT GCG ACT TGG ACA ATG CCT AGC GAT ACT ACA	2555
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820 825 830	
TTG CAG AAT TTA ACG CTA AAT AAC AGT ACG ATC ACG TTA AAT TCA GCT	2603
Leu Gln Asn Leu Thr Leu Asn Asn Ser Thr Ile Thr Leu Asn Ser Ala	
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TAT TCA GCT AGC TCA AAC AAT ACG CCA CGT CGC CGT TCA TTA GAG ACG	2651
Tyr Ser Ala Ser Ser Asn Asn Thr Pro Arg Arg Arg Ser Leu Glu Thr	
850 855 860	
GAA ACA ACG CCA ACA TCG GCA GAA CAT CGT TTC AAC ACA TTG ACA GTA	2699
Glu Thr Thr Pro Thr Ser Ala Glu His Arg Phe Asn Thr Leu Thr Val	
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AAT GGT AAA TTG AGT GGG CAA GGC ACA TTC CAA TTT ACT TCA TCT TTA	2747
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Glu Gln Leu Thr Leu Val Glu Ser Lys Asp Asn Gln Pro Leu Ser Asp	
930 935 940	
AAG CTC AAA TTT ACT TTA GAA AAT GAC CAC GTT GAT GCA GGT GCA TTA	2939
Lys Leu Lys Phe Thr Leu Glu Asn Asp His Val Asp Ala Gly Ala Leu	
945 950 955 960	
CGT TAT AAA TTA GTG AAG AAT GAT GGC GAA TTC CGC TTG CAT AAC CCA	2987
Arg Tyr Lys Leu Val Lys Asn Asp Gly Glu Phe Arg Leu His Asn Pro	
965 970 975	
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Ile Lys Glu Gln Glu Leu His Asn Asp Leu Val Arg Ala Glu Gln Ala	
980 985 990	
GAA CGA ACA TTA GAA GCC AAA CAA GTT GAA CCG ACT GCT AAA ACA CAA	3083
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995 1000 1005	
ACA GGT GAG CCA AAA GTG CGG TCA AGA AGA GCA GCG AGA GCA GCG TTT	3131
Thr Gly Glu Pro Lys Val Arg Ser Arg Arg Ala Ala Arg Ala Ala Phe	
1010 1015 1020	
CCT GAT ACC CTG CCT GAT CAA AGC CTG TTA AAC GCA TTA GAA GCC AAA	3179
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CAA GCT GAA CTG ACT GCT GAA ACA CAA AAA AGT AAG GCA AAA ACA AAA	3227
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CAA TCG GAA AAA GAT CGT CTA GCT CAA GAA GAA GCG GAA AAA CAA CGC	3371
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1125 1130 1135	
CGT CTT TTT GTA GAT CAA GCA CAA TCT GCC GTG TGG ACA AAT ATC GCA	3515
Arg Leu Phe Val Asp Gln Ala Gln Ser Ala Val Trp Thr Asn Ile Ala	
1140 1145 1150	
CAG GAT AAA AGA CGC TAT GAT TCT GAT GCG TTC CGT GCT TAT CAG CAG	3563
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1155 1160 1165	
CAG AAA ACG AAC TTA CGT CAA ATT GGG GTG CAA AAA GCC TTA GCT AAT	3611
Gln Lys Thr Asn Leu Arg Gln Ile Gly Val Gln Lys Ala Leu Ala Asn	
1170 1175 1180	
GGA CGA ATT GGG GCA GTT TTC TCG CAT AGC CGT TCA GAT AAT ACC TTT	3659
Gly Arg Ile Gly Ala Val Phe Ser His Ser Arg Ser Asp Asn Thr Phe	
1185 1190 1195 1200	
GAT GAA CAG GTT AAA AAT CAC GCG ACA TTA ACG ATG ATG TCG GGT TTT	3707
Asp Glu Gln Val Lys Asn His Ala Thr Leu Thr Met Met Ser Gly Phe	
1205 1210 1215	
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1220	1225	1230	
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CGA AAA GCG ATA AAT TAT GGC GTG AAT GCA AGT TAT CAG TTC CGT TTA Arg Lys Ala Ile Asn Tyr Gly Val Asn Ala Ser Tyr Gln Phe Arg Leu 1250 1255 1260			3851
GGG CAA TTG GGC ATT CAG CCT TAT TTT GGA GTT AAT CGC TAT TTT ATT Gly Gln Leu Gly Ile Gln Pro Tyr Phe Gly Val Asn Arg Tyr Phe Ile 1265 1270 1275 1280			3899
GAA CGT GAA AAT TAT CAA TCT GAG GAA GTG AGA GTG AAA ACG CCT AGC Glu Arg Glu Asn Tyr Gln Ser Glu Glu Val Arg Val Lys Thr Pro Ser 1285 1290 1295			3947
CTT GCA TTT AAT CGC TAT AAT GCT GGC ATT CGA GTT GAT TAT ACA TTT Leu Ala Phe Asn Arg Tyr Asn Ala Gly Ile Arg Val Asp Tyr Thr Phe 1300 1305 1310			3995
ACT CCG ACA GAT AAT ATC AGC GTT AAG CCT TAT TTC TTC GTC AAT TAT Thr Pro Thr Asp Asn Ile Ser Val Lys Pro Tyr Phe Phe Val Asn Tyr 1315 1320 1325			4043
GTT GAT GTT TCA AAC GCT AAC GTA CAA ACC ACG GTA AAT CTC ACG GTG Val Asp Val Ser Asn Ala Asn Val Gln Thr Thr Val Asn Leu Thr Val 1330 1335 1340			4091
TTG CAA CAA CCA TTT GGA CGT TAT TGG CAA AAA GAA GTG GGA TTA AAG Leu Gln Gln Pro Phe Gly Arg Tyr Trp Gln Lys Glu Val Gly Leu Lys 1345 1350 1355 1360			4139
GCA GAA ATT TTA CAT TTC CAA ATT TCC GCT TTT ATC TCA AAA TCT CAA Ala Glu Ile Leu His Phe Gln Ile Ser Ala Phe Ile Ser Lys Ser Gln 1365 1370 1375			4187
GGT TCA CAA CTC GGC AAA CAG CAA AAT GTG GGC GTG AAA TTG GGC TAT Gly Ser Gln Leu Gly Lys Gln Gln Asn Val Gly Val Lys Leu Gly Tyr 1380 1385 1390			4235
CGT TGG TAAAAATCAA CATAATTTTA TCGTTTATTG ATAAACAAGG TGGGTCAGAT Arg Trp			4291
CAGATCCCAC CTTTTTTATT CCAATAAT			4319

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1394 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Lys Lys Thr Val Phe Arg Leu Asn Phe Leu Thr Ala Cys Ile Ser
1 5 10 15

Leu Gly Ile Val Ser Gln Ala Trp Ala Gly His Thr Tyr Phe Gly Ile
20 25 30

Asp Tyr Gln Tyr Tyr Arg Asp Phe Ala Glu Asn Lys Gly Lys Phe Thr
35 40 45

Val Gly Ala Gln Asn Ile Lys Val Tyr Asn Lys Gln Gly Gln Leu Val
50 55 60

Gly Thr Ser Met Thr Lys Ala Pro Met Ile Asp Phe Ser Val Val Ser
65 70 75 80

Arg Asn Gly Val Ala Ala Leu Val Glu Asn Gln Tyr Ile Val Ser Val
85 90 95

Ala His Asn Val Gly Tyr Thr Asp Val Asp Phe Gly Ala Glu Gly Asn
100 105 110

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Asn Pro Asp Gln His Arg Phe Thr Tyr Lys Ile Val Lys Arg Asn Asn
 115 120 125
 Tyr Lys Lys Asp Asn Leu His Pro Tyr Glu Asp Asp Tyr His Asn Pro
 130 135 140
 Arg Leu His Lys Phe Val Thr Glu Ala Ala Pro Ile Asp Met Thr Ser
 145 150 155 160
 Asn Met Asn Gly Ser Thr Tyr Ser Asp Arg Thr Lys Tyr Pro Glu Arg
 165 170 175
 Val Arg Ile Gly Ser Gly Arg Gln Phe Trp Arg Asn Asp Gln Asp Lys
 180 185 190
 Gly Asp Gln Val Ala Gly Ala Tyr His Tyr Leu Thr Ala Gly Asn Thr
 195 200 205
 His Asn Gln Arg Gly Ala Gly Asn Gly Tyr Ser Tyr Leu Gly Gly Asp
 210 215 220
 Val Arg Lys Ala Gly Glu Tyr Gly Pro Leu Pro Ile Ala Gly Ser Lys
 225 230 235 240
 Gly Asp Ser Gly Ser Pro Met Phe Ile Tyr Asp Ala Glu Lys Gln Lys
 245 250 255
 Trp Leu Ile Asn Gly Ile Leu Arg Glu Gly Asn Pro Phe Glu Gly Lys
 260 265 270
 Glu Asn Gly Phe Gln Leu Val Arg Lys Ser Tyr Phe Asp Glu Ile Phe
 275 280 285
 Glu Arg Asp Leu His Thr Ser Leu Tyr Thr Arg Ala Gly Asn Gly Val
 290 295 300
 Tyr Thr Ile Ser Gly Asn Asp Asn Gly Gln Gly Ser Ile Thr Gln Lys
 305 310 315 320
 Ser Gly Ile Pro Ser Glu Ile Lys Ile Thr Leu Ala Asn Met Ser Leu
 325 330 335
 Pro Leu Lys Glu Lys Asp Lys Val His Asn Pro Arg Tyr Asp Gly Pro
 340 345 350
 Asn Ile Tyr Ser Pro Arg Leu Asn Asn Gly Glu Thr Leu Tyr Phe Met
 355 360 365
 Asp Gln Lys Gln Gly Ser Leu Ile Phe Ala Ser Asp Ile Asn Gln Gly
 370 375 380
 Ala Gly Gly Leu Tyr Phe Glu Gly Asn Phe Thr Val Ser Pro Asn Ser
 385 390 395 400
 Asn Gln Thr Trp Gln Gly Ala Gly Ile His Val Ser Glu Asn Ser Thr
 405 410 415
 Val Thr Trp Lys Val Asn Gly Val Glu His Asp Arg Leu Ser Lys Ile
 420 425 430
 Gly Lys Gly Thr Leu His Val Gln Ala Lys Gly Glu Asn Lys Gly Ser
 435 440 445
 Ile Ser Val Gly Asp Gly Lys Val Ile Leu Glu Gln Gln Ala Asp Asp
 450 455 460
 Gln Gly Asn Lys Gln Ala Phe Ser Glu Ile Gly Leu Val Ser Gly Arg
 465 470 475 480
 Gly Thr Val Gln Leu Asn Asp Asp Lys Gln Phe Asp Thr Asp Lys Phe
 485 490 495
 Tyr Phe Gly Phe Arg Gly Gly Arg Leu Asp Leu Asn Gly His Ser Leu
 500 505 510
 Thr Phe Lys Arg Ile Gln Asn Thr Asp Glu Gly Ala Met Ile Val Asn
 515 520 525

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His	Asn	Thr	Thr	Gln	Ala	Ala	Asn	Val	Thr	Ile	Thr	Gly	Asn	Glu	Ser		
	530					535						540					
Ile	Val	Leu	Pro	Asn	Gly	Asn	Asn	Ile	Asn	Lys	Leu	Asp	Tyr	Arg	Lys		
545					550					555					560		
Glu	Ile	Ala	Tyr	Asn	Gly	Trp	Phe	Gly	Glu	Thr	Asp	Lys	Asn	Lys	His		
				565					570					575			
Asn	Gly	Arg	Leu	Asn	Leu	Ile	Tyr	Lys	Pro	Thr	Thr	Glu	Asp	Arg	Thr		
			580					585						590			
Leu	Leu	Leu	Ser	Gly	Gly	Thr	Asn	Leu	Lys	Gly	Asp	Ile	Thr	Gln	Thr		
		595					600					605					
Lys	Gly	Lys	Leu	Phe	Phe	Ser	Gly	Arg	Pro	Thr	Pro	His	Ala	Tyr	Asn		
	610					615					620						
His	Leu	Asn	Lys	Arg	Trp	Ser	Glu	Met	Glu	Gly	Ile	Pro	Gln	Gly	Glu		
625					630					635					640		
Ile	Val	Trp	Asp	His	Asp	Trp	Ile	Asn	Arg	Thr	Phe	Lys	Ala	Glu	Asn		
				645					650					655			
Phe	Gln	Ile	Lys	Gly	Gly	Ser	Ala	Val	Val	Ser	Arg	Asn	Val	Ser	Ser		
			660					665					670				
Ile	Glu	Gly	Asn	Trp	Thr	Val	Ser	Asn	Asn	Ala	Asn	Ala	Thr	Phe	Gly		
		675					680					685					
Val	Val	Pro	Asn	Gln	Gln	Asn	Thr	Ile	Cys	Thr	Arg	Ser	Asp	Trp	Thr		
	690					695					700						
Gly	Leu	Thr	Thr	Cys	Gln	Lys	Val	Asp	Leu	Thr	Asp	Thr	Lys	Val	Ile		
705					710					715					720		
Asn	Ser	Ile	Pro	Lys	Thr	Gln	Ile	Asn	Gly	Ser	Ile	Asn	Leu	Thr	Asp		
				725					730					735			
Asn	Ala	Thr	Ala	Asn	Val	Lys	Gly	Leu	Ala	Lys	Leu	Asn	Gly	Asn	Val		
			740					745					750				
Thr	Leu	Thr	Asn	His	Ser	Gln	Phe	Thr	Leu	Ser	Asn	Asn	Ala	Thr	Gln		
		755					760						765				
Ile	Gly	Asn	Ile	Arg	Leu	Ser	Asp	Asn	Ser	Thr	Ala	Thr	Val	Asp	Asn		
	770					775					780						
Ala	Asn	Leu	Asn	Gly	Asn	Val	His	Leu	Thr	Asp	Ser	Ala	Gln	Phe	Ser		
785					790					795					800		
Leu	Lys	Asn	Ser	His	Phe	Ser	His	Gln	Ile	Gln	Gly	Asp	Lys	Gly	Thr		
				805					810					815			
Thr	Val	Thr	Leu	Glu	Asn	Ala	Thr	Trp	Thr	Met	Pro	Ser	Asp	Thr	Thr		
			820					825						830			
Leu	Gln	Asn	Leu	Thr	Leu	Asn	Asn	Ser	Thr	Ile	Thr	Leu	Asn	Ser	Ala		
		835					840					845					
Tyr	Ser	Ala	Ser	Ser	Asn	Asn	Thr	Pro	Arg	Arg	Arg	Ser	Leu	Glu	Thr		
	850					855						860					
Glu	Thr	Thr	Pro	Thr	Ser	Ala	Glu	His	Arg	Phe	Asn	Thr	Leu	Thr	Val		
865					870					875					880		
Asn	Gly	Lys	Leu	Ser	Gly	Gln	Gly	Thr	Phe	Gln	Phe	Thr	Ser	Ser	Leu		
				885					890					895			
Phe	Gly	Tyr	Lys	Ser	Asp	Lys	Leu	Lys	Leu	Ser	Asn	Asp	Ala	Glu	Gly		
			900					905					910				
Asp	Tyr	Ile	Leu	Ser	Val	Arg	Asn	Thr	Gly	Lys	Glu	Pro	Glu	Thr	Leu		
		915					920						925				
Glu	Gln	Leu	Thr	Leu	Val	Glu	Ser	Lys	Asp	Asn	Gln	Pro	Leu	Ser	Asp		
	930					935					940						
Lys	Leu	Lys	Phe	Thr	Leu	Glu	Asn	Asp	His	Val	Asp	Ala	Gly	Ala	Leu		

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945	950	955	960
Arg Tyr Lys Leu Val Lys Asn Asp Gly Glu Phe Arg Leu His Asn Pro 965 970 975			
Ile Lys Glu Gln Glu Leu His Asn Asp Leu Val Arg Ala Glu Gln Ala 980 985 990			
Glu Arg Thr Leu Glu Ala Lys Gln Val Glu Pro Thr Ala Lys Thr Gln 995 1000 1005			
Thr Gly Glu Pro Lys Val Arg Ser Arg Arg Ala Ala Arg Ala Ala Phe 1010 1015 1020			
Pro Asp Thr Leu Pro Asp Gln Ser Leu Leu Asn Ala Leu Glu Ala Lys 1025 1030 1035 1040			
Gln Ala Glu Leu Thr Ala Glu Thr Gln Lys Ser Lys Ala Lys Thr Lys 1045 1050 1055			
Lys Val Arg Ser Lys Arg Ala Val Phe Ser Asp Pro Leu Leu Asp Gln 1060 1065 1070			
Ser Leu Phe Ala Leu Glu Ala Ala Leu Glu Val Ile Asp Ala Pro Gln 1075 1080 1085			
Gln Ser Glu Lys Asp Arg Leu Ala Gln Glu Glu Ala Glu Lys Gln Arg 1090 1095 1100			
Lys Gln Lys Asp Leu Ile Ser Arg Tyr Ser Asn Ser Ala Leu Ser Glu 1105 1110 1115 1120			
Leu Ser Ala Thr Val Asn Ser Met Leu Ser Val Gln Asp Glu Leu Asp 1125 1130 1135			
Arg Leu Phe Val Asp Gln Ala Gln Ser Ala Val Trp Thr Asn Ile Ala 1140 1145 1150			
Gln Asp Lys Arg Arg Tyr Asp Ser Asp Ala Phe Arg Ala Tyr Gln Gln 1155 1160 1165			
Gln Lys Thr Asn Leu Arg Gln Ile Gly Val Gln Lys Ala Leu Ala Asn 1170 1175 1180			
Gly Arg Ile Gly Ala Val Phe Ser His Ser Arg Ser Asp Asn Thr Phe 1185 1190 1195 1200			
Asp Glu Gln Val Lys Asn His Ala Thr Leu Thr Met Met Ser Gly Phe 1205 1210 1215			
Ala Gln Tyr Gln Trp Gly Asp Leu Gln Phe Gly Val Asn Val Gly Thr 1220 1225 1230			
Gly Ile Ser Ala Ser Lys Met Ala Glu Glu Gln Ser Arg Lys Ile His 1235 1240 1245			
Arg Lys Ala Ile Asn Tyr Gly Val Asn Ala Ser Tyr Gln Phe Arg Leu 1250 1255 1260			
Gly Gln Leu Gly Ile Gln Pro Tyr Phe Gly Val Asn Arg Tyr Phe Ile 1265 1270 1275 1280			
Glu Arg Glu Asn Tyr Gln Ser Glu Glu Val Arg Val Lys Thr Pro Ser 1285 1290 1295			
Leu Ala Phe Asn Arg Tyr Asn Ala Gly Ile Arg Val Asp Tyr Thr Phe 1300 1305 1310			
Thr Pro Thr Asp Asn Ile Ser Val Lys Pro Tyr Phe Phe Val Asn Tyr 1315 1320 1325			
Val Asp Val Ser Asn Ala Asn Val Gln Thr Thr Val Asn Leu Thr Val 1330 1335 1340			
Leu Gln Gln Pro Phe Gly Arg Tyr Trp Gln Lys Glu Val Gly Leu Lys 1345 1350 1355 1360			
Ala Glu Ile Leu His Phe Gln Ile Ser Ala Phe Ile Ser Lys Ser Gln 1365 1370 1375			

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Gly Ser Gln Leu Gly Lys Gln Gln Asn Val Gly Val Lys Leu Gly Tyr
 1380 1385 1390

Arg Trp

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1541 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Leu Asn Lys Lys Phe Lys Leu Asn Phe Ile Ala Leu Thr Val Ala
 1 5 10 15
 Tyr Ala Leu Thr Pro Tyr Thr Glu Ala Ala Leu Val Arg Asp Asp Val
 20 25 30
 Asp Tyr Gln Ile Phe Arg Asp Phe Ala Glu Asn Lys Gly Lys Phe Ser
 35 40 45
 Val Gly Ala Thr Asn Val Leu Val Lys Asp Lys Asn Asn Lys Asp Leu
 50 55 60
 Gly Thr Ala Leu Pro Asn Gly Ile Pro Met Ile Asp Phe Ser Val Val
 65 70 75 80
 Asp Val Asp Lys Arg Ile Ala Thr Leu Ile Asn Pro Gln Tyr Val Val
 85 90 95
 Gly Val Lys His Val Ser Asn Gly Val Ser Glu Leu His Phe Gly Asn
 100 105 110
 Leu Asn Gly Asn Met Asn Asn Gly Asn Ala Lys Ala His Arg Asp Val
 115 120 125
 Ser Ser Glu Glu Asn Arg Tyr Phe Ser Val Glu Lys Asn Glu Tyr Pro
 130 135 140
 Thr Lys Leu Asn Gly Lys Thr Val Thr Thr Glu Asp Gln Thr Gln Lys
 145 150 155 160
 Arg Arg Glu Asp Tyr Tyr Met Pro Arg Leu Asp Lys Phe Val Thr Glu
 165 170 175
 Val Ala Pro Ile Glu Ala Ser Thr Ala Ser Ser Asp Ala Gly Thr Tyr
 180 185 190
 Asn Asp Gln Asn Lys Tyr Pro Ala Phe Val Arg Leu Gly Ser Gly Ser
 195 200 205
 Gln Phe Ile Tyr Lys Lys Gly Asp Asn Tyr Ser Leu Ile Leu Asn Asn
 210 215 220
 His Glu Val Gly Gly Asn Asn Leu Lys Leu Val Gly Asp Ala Tyr Thr
 225 230 235 240
 Tyr Gly Ile Ala Gly Thr Pro Tyr Lys Val Asn His Glu Asn Asn Gly
 245 250 255
 Leu Ile Gly Phe Gly Asn Ser Lys Glu Glu His Ser Asp Pro Lys Gly
 260 265 270
 Ile Leu Ser Gln Asp Pro Leu Thr Asn Tyr Ala Val Leu Gly Asp Ser
 275 280 285
 Gly Ser Pro Leu Phe Val Tyr Asp Arg Glu Lys Gly Lys Trp Leu Phe
 290 295 300
 Leu Gly Ser Tyr Asp Phe Trp Ala Gly Tyr Asn Lys Lys Ser Trp Gln
 305 310 315 320
 Glu Trp Asn Ile Tyr Lys Ser Gln Phe Thr Lys Asp Val Leu Asn Lys
 325 330 335

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Asp	Ser	Ala	Gly	Ser	Leu	Ile	Gly	Ser	Lys	Thr	Asp	Tyr	Ser	Trp	Ser
			340					345					350		
Ser	Asn	Gly	Lys	Thr	Ser	Thr	Ile	Thr	Gly	Gly	Glu	Lys	Ser	Leu	Asn
		355					360					365			
Val	Asp	Leu	Ala	Asp	Gly	Lys	Asp	Lys	Pro	Asn	His	Gly	Lys	Ser	Val
	370					375					380				
Thr	Phe	Glu	Gly	Ser	Gly	Thr	Leu	Thr	Leu	Asn	Asn	Asn	Ile	Asp	Gln
385					390					395					400
Gly	Ala	Gly	Gly	Leu	Phe	Phe	Glu	Gly	Asp	Tyr	Glu	Val	Lys	Gly	Thr
				405					410					415	
Ser	Asp	Asn	Thr	Thr	Trp	Lys	Gly	Ala	Gly	Val	Ser	Val	Ala	Glu	Gly
			420					425					430		
Lys	Thr	Val	Thr	Trp	Lys	Val	His	Asn	Pro	Gln	Tyr	Asp	Arg	Leu	Ala
		435					440					445			
Lys	Ile	Gly	Lys	Gly	Thr	Leu	Ile	Val	Glu	Gly	Thr	Gly	Asp	Asn	Lys
	450					455					460				
Gly	Ser	Leu	Lys	Val	Gly	Asp	Gly	Thr	Val	Ile	Leu	Lys	Gln	Gln	Thr
465					470					475					480
Asn	Gly	Ser	Gly	Gln	His	Ala	Phe	Ala	Ser	Val	Gly	Ile	Val	Ser	Gly
				485					490					495	
Arg	Ser	Thr	Leu	Val	Leu	Asn	Asp	Asp	Lys	Gln	Val	Asp	Pro	Asn	Ser
			500					505					510		
Ile	Tyr	Phe	Gly	Phe	Arg	Gly	Gly	Arg	Leu	Asp	Leu	Asn	Gly	Asn	Ser
		515					520					525			
Leu	Thr	Phe	Asp	His	Ile	Arg	Asn	Ile	Asp	Asp	Gly	Ala	Arg	Leu	Val
	530					535					540				
Asn	His	Asn	Met	Thr	Asn	Ala	Ser	Asn	Ile	Thr	Ile	Thr	Gly	Glu	Ser
545					550					555					560
Leu	Ile	Thr	Asp	Pro	Asn	Thr	Ile	Thr	Pro	Tyr	Asn	Ile	Asp	Ala	Pro
				565					570					575	
Asp	Glu	Asp	Asn	Pro	Tyr	Ala	Phe	Arg	Arg	Ile	Lys	Asp	Gly	Gly	Gln
			580					585					590		
Leu	Tyr	Leu	Asn	Leu	Glu	Asn	Tyr	Thr	Tyr	Tyr	Ala	Leu	Arg	Lys	Gly
		595					600					605			
Ala	Ser	Thr	Arg	Ser	Glu	Leu	Pro	Lys	Asn	Ser	Gly	Glu	Ser	Asn	Glu
						615					620				
Asn	Trp	Leu	Tyr	Met	Gly	Lys	Thr	Ser	Asp	Glu	Ala	Lys	Arg	Asn	Val
625					630					635					640
Met	Asn	His	Ile	Asn	Asn	Glu	Arg	Met	Asn	Gly	Phe	Asn	Gly	Tyr	Phe
				645					650					655	
Gly	Glu	Glu	Glu	Gly	Lys	Asn	Asn	Gly	Asn	Leu	Asn	Val	Thr	Phe	Lys
				660				665					670		
Gly	Lys	Ser	Glu	Gln	Asn	Arg	Phe	Leu	Leu	Thr	Gly	Gly	Thr	Asn	Leu
			675				680					685			
Asn	Gly	Asp	Leu	Thr	Val	Glu	Lys	Gly	Thr	Leu	Phe	Leu	Ser	Gly	Arg
	690					695					700				
Pro	Thr	Pro	His	Ala	Arg	Asp	Ile	Ala	Gly	Ile	Ser	Ser	Thr	Lys	Lys
705					710					715					720
Asp	Pro	His	Phe	Ala	Glu	Asn	Asn	Glu	Val	Val	Val	Glu	Asp	Asp	Trp
				725					730					735	
Ile	Asn	Arg	Asn	Phe	Lys	Ala	Thr	Thr	Met	Asn	Val	Thr	Gly	Asn	Ala
			740					745					750		
Ser	Leu	Tyr	Ser	Gly	Arg	Asn	Val	Ala	Asn	Ile	Thr	Ser	Asn	Ile	Thr

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755				760				765							
Ala	Ser	Asn	Lys	Ala	Gln	Val	His	Ile	Gly	Tyr	Lys	Thr	Gly	Asp	Thr
	770				775						780				
Val	Cys	Val	Arg	Ser	Asp	Tyr	Thr	Gly	Tyr	Val	Thr	Cys	Thr	Thr	Asp
	785				790					795					800
Lys	Leu	Ser	Asp	Lys	Ala	Leu	Asn	Ser	Phe	Asn	Pro	Thr	Asn	Leu	Arg
				805					810					815	
Gly	Asn	Val	Asn	Leu	Thr	Glu	Ser	Ala	Asn	Phe	Val	Leu	Gly	Lys	Ala
			820					825					830		
Asn	Leu	Phe	Gly	Thr	Ile	Gln	Ser	Arg	Gly	Asn	Ser	Gln	Val	Arg	Leu
		835					840					845			
Thr	Glu	Asn	Ser	His	Trp	His	Leu	Thr	Gly	Asn	Ser	Asp	Val	His	Gln
	850					855					860				
Leu	Asp	Leu	Ala	Asn	Gly	His	Ile	His	Leu	Asn	Ser	Ala	Asp	Asn	Ser
	865				870					875					880
Asn	Asn	Val	Thr	Lys	Tyr	Asn	Thr	Leu	Thr	Val	Asn	Ser	Leu	Ser	Gly
				885					890					895	
Asn	Gly	Ser	Phe	Tyr	Tyr	Leu	Thr	Asp	Leu	Ser	Asn	Lys	Gln	Gly	Asp
			900					905					910		
Lys	Val	Val	Val	Thr	Lys	Ser	Ala	Thr	Gly	Asn	Phe	Thr	Leu	Gln	Val
		915					920						925		
Ala	Asp	Lys	Thr	Gly	Glu	Pro	Asn	His	Asn	Glu	Leu	Thr	Leu	Phe	Asp
	930					935					940				
Ala	Ser	Lys	Ala	Gln	Arg	Asp	His	Leu	Asn	Val	Ser	Leu	Val	Gly	Asn
	945				950					955					960
Thr	Val	Asp	Leu	Gly	Ala	Trp	Lys	Tyr	Lys	Leu	Arg	Asn	Val	Asn	Gly
			965						970					975	
Arg	Tyr	Asp	Leu	Tyr	Asn	Pro	Glu	Val	Glu	Lys	Arg	Asn	Gln	Thr	Val
		980						985					990		
Asp	Thr	Thr	Asn	Ile	Thr	Thr	Pro	Asn	Asn	Ile	Gln	Ala	Asp	Val	Pro
		995					1000					1005			
Ser	Val	Pro	Ser	Asn	Asn	Glu	Glu	Ile	Ala	Arg	Val	Asp	Glu	Ala	Pro
	1010					1015					1020				
Val	Pro	Pro	Pro	Ala	Pro	Ala	Thr	Pro	Ser	Glu	Thr	Thr	Glu	Thr	Val
	1025				1030					1035					1040
Ala	Glu	Asn	Ser	Lys	Gln	Glu	Ser	Lys	Thr	Val	Glu	Lys	Asn	Glu	Gln
				1045					1050				1055		
Asp	Ala	Thr	Glu	Thr	Thr	Ala	Gln	Asn	Arg	Glu	Val	Ala	Lys	Glu	Ala
		1060						1065					1070		
Lys	Ser	Asn	Val	Lys	Ala	Asn	Thr	Gln	Thr	Asn	Glu	Val	Ala	Gln	Ser
		1075					1080					1085			
Gly	Ser	Glu	Thr	Lys	Glu	Thr	Gln	Thr	Thr	Glu	Thr	Lys	Glu	Thr	Ala
	1090					1095					1100				
Thr	Val	Glu	Lys	Glu	Glu	Lys	Ala	Lys	Val	Glu	Thr	Glu	Lys	Thr	Gln
	1105				1110					1115					1120
Glu	Val	Pro	Lys	Val	Thr	Ser	Gln	Val	Ser	Pro	Lys	Gln	Glu	Gln	Ser
				1125					1130					1135	
Glu	Thr	Val	Gln	Pro	Gln	Ala	Glu	Pro	Ala	Arg	Glu	Asn	Asp	Pro	Thr
			1140					1145					1150		
Val	Asn	Ile	Lys	Glu	Pro	Gln	Ser	Gln	Thr	Asn	Thr	Thr	Ala	Asp	Thr
		1155					1160						1165		
Glu	Gln	Pro	Ala	Lys	Glu	Thr	Ser	Ser	Asn	Val	Glu	Gln	Pro	Val	Thr
						1175					1180				

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Glu Ser Thr Thr Val Asn Thr Gly Asn Ser Val Val Glu Asn Pro Glu
 1185 1190 1195 1200
 Asn Thr Thr Pro Ala Thr Thr Gln Pro Thr Val Asn Ser Glu Ser Ser
 1205 1210 1215
 Asn Lys Pro Lys Asn Arg His Arg Arg Ser Val Arg Ser Val Pro His
 1220 1225 1230
 Asn Val Glu Pro Ala Thr Thr Ser Ser Asn Asp Arg Ser Thr Val Ala
 1235 1240 1245
 Leu Cys Asp Leu Thr Ser Thr Asn Thr Asn Ala Val Leu Ser Asp Ala
 1250 1255 1260
 Arg Ala Lys Ala Gln Phe Val Ala Leu Asn Val Gly Lys Ala Val Ser
 1265 1270 1275 1280
 Gln His Ile Ser Gln Leu Glu Met Asn Asn Glu Gly Gln Tyr Asn Val
 1285 1290 1295
 Trp Val Ser Asn Thr Ser Met Asn Lys Asn Tyr Ser Ser Ser Gln Tyr
 1300 1305 1310
 Arg Arg Phe Ser Ser Lys Ser Thr Gln Thr Gln Leu Gly Trp Asp Gln
 1315 1320 1325
 Thr Ile Ser Asn Asn Val Gln Leu Gly Gly Val Phe Thr Tyr Val Arg
 1330 1335 1340
 Asn Ser Asn Asn Phe Asp Lys Ala Thr Ser Lys Asn Thr Leu Ala Gln
 1345 1350 1355 1360
 Val Asn Phe Tyr Ser Lys Tyr Tyr Ala Asp Asn His Trp Tyr Leu Gly
 1365 1370 1375
 Ile Asp Leu Gly Tyr Gly Lys Phe Gln Ser Lys Leu Gln Thr Asn His
 1380 1385 1390
 Asn Ala Lys Phe Ala Arg His Thr Ala Gln Phe Gly Leu Thr Ala Gly
 1395 1400 1405
 Lys Ala Phe Asn Leu Gly Asn Phe Gly Ile Thr Pro Ile Val Gly Val
 1410 1415 1420
 Arg Tyr Ser Tyr Leu Ser Asn Ala Asp Phe Ala Leu Asp Gln Ala Arg
 1425 1430 1435 1440
 Ile Lys Val Asn Pro Ile Ser Val Lys Thr Ala Phe Ala Gln Val Asp
 1445 1450 1455
 Leu Ser Tyr Thr Tyr His Leu Gly Glu Phe Ser Val Thr Pro Ile Leu
 1460 1465 1470
 Ser Ala Arg Tyr Asp Ala Asn Gln Gly Ser Gly Lys Ile Asn Val Asn
 1475 1480 1485
 Gly Tyr Asp Phe Ala Tyr Asn Val Glu Asn Gln Gln Gln Tyr Asn Ala
 1490 1495 1500
 Gly Leu Lys Leu Lys Tyr His Asn Val Lys Leu Ser Leu Ile Gly Gly
 1505 1510 1515 1520
 Leu Thr Lys Ala Lys Gln Ala Glu Lys Gln Lys Thr Ala Glu Leu Lys
 1525 1530 1535
 Leu Ser Phe Ser Phe
 1540

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1545 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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Met Leu Asn Lys Lys Phe Lys Leu Asn Phe Ile Ala Leu Thr Val Ala
 1 5 10 15
 Tyr Ala Leu Thr Pro Tyr Thr Glu Ala Ala Leu Val Arg Asp Asp Val
 20 25 30
 Asp Tyr Gln Ile Phe Arg Asp Phe Ala Glu Asn Lys Gly Lys Phe Ser
 35 40 45
 Val Gly Ala Thr Asn Val Glu Val Arg Asp Lys Asn Asn Arg Pro Leu
 50 55 60
 Gly Asn Val Leu Pro Asn Gly Ile Pro Met Ile Asp Phe Ser Val Val
 65 70 75 80
 Asp Val Asp Lys Arg Ile Ala Thr Leu Val Asn Pro Gln Tyr Val Val
 85 90 95
 Gly Val Lys His Val Ser Asn Gly Val Ser Glu Leu His Phe Gly Asn
 100 105 110
 Leu Asn Gly Asn Met Asn Asn Gly Asn Ala Lys Ala His Arg Asp Val
 115 120 125
 Ser Ser Glu Glu Asn Arg Tyr Tyr Thr Val Glu Lys Asn Glu Tyr Pro
 130 135 140
 Thr Lys Leu Asn Gly Lys Ala Val Thr Thr Glu Asp Gln Ala Gln Lys
 145 150 155 160
 Arg Arg Glu Asp Tyr Tyr Met Pro Arg Leu Asp Lys Phe Val Thr Glu
 165 170 175
 Val Ala Pro Ile Glu Ala Ser Thr Asp Ser Ser Thr Ala Gly Thr Tyr
 180 185 190
 Asn Asn Lys Asp Lys Tyr Pro Tyr Phe Val Arg Leu Gly Ser Gly Thr
 195 200 205
 Gln Phe Ile Tyr Glu Asn Gly Thr Arg Tyr Glu Leu Trp Leu Gly Lys
 210 215 220
 Glu Gly Gln Lys Ser Asp Ala Gly Gly Tyr Asn Leu Lys Leu Val Gly
 225 230 235 240
 Asn Ala Tyr Thr Tyr Gly Ile Ala Gly Thr Pro Tyr Glu Val Asn His
 245 250 255
 Glu Asn Asp Gly Leu Ile Gly Phe Gly Asn Ser Asn Asn Glu Tyr Ile
 260 265 270
 Asn Pro Lys Glu Ile Leu Ser Lys Lys Pro Leu Thr Asn Tyr Ala Val
 275 280 285
 Leu Gly Asp Ser Gly Ser Pro Leu Phe Val Tyr Asp Arg Glu Lys Gly
 290 295 300
 Lys Trp Leu Phe Leu Gly Ser Tyr Asp Tyr Trp Ala Gly Tyr Asn Lys
 305 310 315 320
 Lys Ser Trp Gln Glu Trp Asn Ile Tyr Lys Pro Glu Phe Ala Glu Lys
 325 330 335
 Ile Tyr Glu Gln Tyr Ser Ala Gly Ser Leu Ile Gly Ser Lys Thr Asp
 340 345 350
 Tyr Ser Trp Ser Ser Asn Gly Lys Thr Ser Thr Ile Thr Gly Gly Glu
 355 360 365
 Lys Ser Leu Asn Val Asp Leu Ala Asp Gly Lys Asp Lys Pro Asn His
 370 375 380
 Gly Lys Ser Val Thr Phe Glu Gly Ser Gly Thr Leu Thr Leu Asn Asn
 385 390 395 400
 Asn Ile Asp Gln Gly Ala Gly Gly Leu Phe Phe Glu Gly Asp Tyr Glu
 405 410 415

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Val	Lys	Gly	Thr	Ser	Asp	Asn	Thr	Thr	Trp	Lys	Gly	Ala	Gly	Val	Ser
			420					425					430		
Val	Ala	Glu	Gly	Lys	Thr	Val	Thr	Trp	Lys	Val	His	Asn	Pro	Gln	Tyr
		435					440					445			
Asp	Arg	Leu	Ala	Lys	Ile	Gly	Lys	Gly	Thr	Leu	Ile	Val	Glu	Gly	Thr
	450					455					460				
Gly	Asp	Asn	Lys	Gly	Ser	Leu	Lys	Val	Gly	Asp	Gly	Thr	Val	Ile	Leu
465					470					475					480
Lys	Gln	Gln	Thr	Asn	Gly	Ser	Gly	Gln	His	Ala	Phe	Ala	Ser	Val	Gly
				485					490					495	
Ile	Val	Ser	Gly	Arg	Ser	Thr	Leu	Val	Leu	Asn	Asp	Asp	Lys	Gln	Val
			500					505					510		
Asp	Pro	Asn	Ser	Ile	Tyr	Phe	Gly	Phe	Arg	Gly	Gly	Arg	Leu	Asp	Leu
		515					520					525			
Asn	Gly	Asn	Ser	Leu	Thr	Phe	Asp	His	Ile	Arg	Asn	Ile	Asp	Glu	Gly
	530						535				540				
Ala	Arg	Leu	Val	Asn	His	Ser	Thr	Ser	Lys	His	Ser	Thr	Val	Thr	Ile
545					550					555					560
Thr	Gly	Asp	Asn	Leu	Ile	Thr	Asp	Pro	Asn	Asn	Val	Ser	Ile	Tyr	Tyr
				565					570					575	
Val	Lys	Pro	Leu	Glu	Asp	Asp	Asn	Pro	Tyr	Ala	Ile	Arg	Gln	Ile	Lys
			580					585					590		
Tyr	Gly	Tyr	Gln	Leu	Tyr	Phe	Asn	Glu	Glu	Asn	Arg	Thr	Tyr	Tyr	Ala
		595					600					605			
Leu	Lys	Lys	Asp	Ala	Ser	Ile	Arg	Ser	Glu	Phe	Pro	Gln	Asn	Arg	Gly
	610						615				620				
Glu	Ser	Asn	Asn	Ser	Trp	Leu	Tyr	Met	Gly	Thr	Glu	Lys	Ala	Asp	Ala
625					630					635					640
Gln	Lys	Asn	Ala	Met	Asn	His	Ile	Asn	Asn	Glu	Arg	Met	Asn	Gly	Phe
				645					650					655	
Asn	Gly	Tyr	Phe	Gly	Glu	Glu	Glu	Gly	Lys	Asn	Asn	Gly	Asn	Leu	Asn
			660					665					670		
Val	Thr	Phe	Lys	Gly	Lys	Ser	Glu	Gln	Asn	Arg	Phe	Leu	Leu	Thr	Gly
		675					680					685			
Gly	Thr	Asn	Leu	Asn	Gly	Asp	Leu	Asn	Val	Gln	Gln	Gly	Thr	Leu	Phe
	690					695					700				
Leu	Ser	Gly	Arg	Pro	Thr	Pro	His	Ala	Arg	Asp	Ile	Ala	Gly	Ile	Ser
705					710					715					720
Ser	Thr	Lys	Lys	Asp	Ser	His	Phe	Ser	Glu	Asn	Asn	Glu	Val	Val	Val
				725					730					735	
Glu	Asp	Asp	Trp	Ile	Asn	Arg	Asn	Phe	Lys	Ala	Thr	Asn	Ile	Asn	Val
			740					745					750		
Thr	Asn	Asn	Ala	Thr	Leu	Tyr	Ser	Gly	Arg	Asn	Val	Glu	Ser	Ile	Thr
			755				760					765			
Ser	Asn	Ile	Thr	Ala	Ser	Asn	Asn	Ala	Lys	Val	His	Ile	Gly	Tyr	Lys
	770					775					780				
Ala	Gly	Asp	Thr	Val	Cys	Val	Arg	Ser	Asp	Tyr	Thr	Gly	Tyr	Val	Thr
785					790					795					800
Cys	Thr	Thr	Asp	Lys	Leu	Ser	Asp	Lys	Ala	Leu	Asn	Ser	Phe	Asn	Pro
				805					810					815	
Thr	Asn	Leu	Arg	Gly	Asn	Val	Asn	Leu	Thr	Glu	Ser	Ala	Asn	Phe	Val
			820					825					830		
Leu	Gly	Lys	Ala	Asn	Leu	Phe	Gly	Thr	Ile	Gln	Ser	Arg	Gly	Asn	Ser

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835					840					845					
Gln	Val	Arg	Leu	Thr	Glu	Asn	Ser	His	Trp	His	Leu	Thr	Gly	Asn	Ser
	850					855					860				
Asp	Val	His	Gln	Leu	Asp	Leu	Ala	Asn	Gly	His	Ile	His	Leu	Asn	Ser
	865					870					875				880
Ala	Asp	Asn	Ser	Asn	Asn	Val	Thr	Lys	Tyr	Asn	Thr	Leu	Thr	Val	Asn
				885					890						895
Ser	Leu	Ser	Gly	Asn	Gly	Ser	Phe	Tyr	Tyr	Leu	Thr	Asp	Leu	Ser	Asn
			900					905					910		
Lys	Gln	Gly	Asp	Lys	Val	Val	Val	Thr	Lys	Ser	Ala	Thr	Gly	Asn	Phe
		915						920					925		
Thr	Leu	Gln	Val	Ala	Asp	Lys	Thr	Gly	Glu	Pro	Asn	His	Asn	Glu	Leu
	930					935						940			
Thr	Leu	Phe	Asp	Ala	Ser	Lys	Ala	Gln	Arg	Asp	His	Leu	Asn	Val	Ser
	945					950						955			960
Leu	Val	Gly	Asn	Thr	Val	Asp	Leu	Gly	Ala	Trp	Lys	Tyr	Lys	Leu	Arg
				965					970						975
Asn	Val	Asn	Gly	Arg	Tyr	Asp	Leu	Tyr	Asn	Pro	Glu	Val	Glu	Lys	Arg
			980					985					990		
Asn	Gln	Thr	Val	Asp	Thr	Thr	Asn	Ile	Thr	Thr	Pro	Asn	Asn	Ile	Gln
		995					1000						1005		
Ala	Asp	Val	Pro	Ser	Val	Pro	Ser	Asn	Asn	Glu	Glu	Ile	Ala	Arg	Val
	1010						1015						1020		
Asp	Glu	Ala	Pro	Val	Pro	Pro	Pro	Ala	Pro	Ala	Thr	Pro	Ser	Glu	Thr
	1025						1030					1035			1040
Thr	Glu	Thr	Val	Ala	Glu	Asn	Ser	Lys	Gln	Glu	Ser	Lys	Thr	Val	Glu
				1045					1050						1055
Lys	Asn	Glu	Gln	Asp	Ala	Thr	Glu	Thr	Thr	Ala	Gln	Asn	Arg	Glu	Val
			1060					1065					1070		
Ala	Lys	Glu	Ala	Lys	Ser	Asn	Val	Lys	Ala	Asn	Thr	Gln	Thr	Asn	Glu
		1075					1080						1085		
Val	Ala	Gln	Ser	Gly	Ser	Glu	Thr	Lys	Glu	Thr	Gln	Thr	Thr	Glu	Thr
	1090						1095						1100		
Lys	Glu	Thr	Ala	Thr	Val	Glu	Lys	Glu	Glu	Lys	Ala	Lys	Val	Glu	Thr
	1105						1110					1115			1120
Glu	Lys	Thr	Gln	Glu	Val	Pro	Lys	Val	Thr	Ser	Gln	Val	Ser	Pro	Lys
				1125					1130						1135
Gln	Glu	Gln	Ser	Glu	Thr	Val	Gln	Pro	Gln	Ala	Glu	Pro	Ala	Arg	Glu
				1140				1145						1150	
Asn	Asp	Pro	Thr	Val	Asn	Ile	Lys	Glu	Pro	Gln	Ser	Gln	Thr	Asn	Thr
		1155					1160						1165		
Thr	Ala	Asp	Thr	Glu	Gln	Pro	Ala	Lys	Glu	Thr	Ser	Ser	Asn	Val	Glu
	1170						1175					1180			
Gln	Pro	Val	Thr	Glu	Ser	Thr	Thr	Val	Asn	Thr	Gly	Asn	Ser	Val	Val
	1185						1190					1195			1200
Glu	Asn	Pro	Glu	Asn	Thr	Thr	Pro	Ala	Thr	Thr	Gln	Pro	Thr	Val	Asn
				1205					1210						1215
Ser	Glu	Ser	Ser	Asn	Lys	Pro	Lys	Asn	Arg	His	Arg	Arg	Ser	Val	Arg
				1220				1225						1230	
Ser	Val	Pro	His	Asn	Val	Glu	Pro	Ala	Thr	Thr	Ser	Ser	Asn	Asp	Arg
		1235						1240						1245	
Ser	Thr	Val	Ala	Leu	Cys	Asp	Leu	Thr	Ser	Thr	Asn	Thr	Asn	Ala	Val
	1250						1255						1260		

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Leu Ser Asp Ala Arg Ala Lys Ala Gln Phe Val Ala Leu Asn Val Gly
 1265 1270 1275 1280
 Lys Ala Val Ser Gln His Ile Ser Gln Leu Glu Met Asn Asn Glu Gly
 1285 1290 1295
 Gln Tyr Asn Val Trp Val Ser Asn Thr Ser Met Asn Lys Asn Tyr Ser
 1300 1305 1310
 Ser Ser Gln Tyr Arg Arg Phe Ser Ser Lys Ser Thr Gln Thr Gln Leu
 1315 1320 1325
 Gly Trp Asp Gln Thr Ile Ser Asn Asn Val Gln Leu Gly Gly Val Phe
 1330 1335 1340
 Thr Tyr Val Arg Asn Ser Asn Asn Phe Asp Lys Ala Thr Ser Lys Asn
 1345 1350 1355 1360
 Thr Leu Ala Gln Val Asn Phe Tyr Ser Lys Tyr Tyr Ala Asp Asn His
 1365 1370 1375
 Trp Tyr Leu Gly Ile Asp Leu Gly Tyr Gly Lys Phe Gln Ser Lys Leu
 1380 1385 1390
 Gln Thr Asn His Asn Ala Lys Phe Ala Arg His Thr Ala Gln Phe Gly
 1395 1400 1405
 Leu Thr Ala Gly Lys Ala Phe Asn Leu Gly Asn Phe Gly Ile Thr Pro
 1410 1415 1420
 Ile Val Gly Val Arg Tyr Ser Tyr Leu Ser Asn Ala Asp Phe Ala Leu
 1425 1430 1435 1440
 Asp Gln Ala Arg Ile Lys Val Asn Pro Ile Ser Val Lys Thr Ala Phe
 1445 1450 1455
 Ala Gln Val Asp Leu Ser Tyr Thr Tyr His Leu Gly Glu Phe Ser Val
 1460 1465 1470
 Thr Pro Ile Leu Ser Ala Arg Tyr Asp Ala Asn Gln Gly Ser Gly Lys
 1475 1480 1485
 Ile Asn Val Asn Gly Tyr Asp Phe Ala Tyr Asn Val Glu Asn Gln Gln
 1490 1495 1500
 Gln Tyr Asn Ala Gly Leu Lys Leu Lys Tyr His Asn Val Lys Leu Ser
 1505 1510 1515 1520
 Leu Ile Gly Gly Leu Thr Lys Ala Lys Gln Ala Glu Lys Gln Lys Thr
 1525 1530 1535
 Ala Glu Leu Lys Leu Ser Phe Ser Phe
 1540 1545

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1702 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Leu Asn Lys Lys Phe Lys Leu Asn Phe Ile Ala Leu Thr Val Ala
 1 5 10 15
 Tyr Ala Leu Thr Pro Tyr Thr Glu Ala Ala Leu Val Arg Asp Asp Val
 20 25 30
 Asp Tyr Gln Ile Phe Arg Asp Phe Ala Glu Asn Lys Gly Arg Phe Ser
 35 40 45
 Val Gly Ala Thr Asn Val Glu Val Arg Asp Lys Asn Asn His Ser Leu
 50 55 60
 Gly Asn Val Leu Pro Asn Gly Ile Pro Met Ile Asp Phe Ser Val Val
 65 70 75 80

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Asp Val Asp Lys Arg Ile Ala Thr Leu Ile Asn Pro Gln Tyr Val Val
 85 90 95
 Gly Val Lys His Val Ser Asn Gly Val Ser Glu Leu His Phe Gly Asn
 100 105 110
 Leu Asn Gly Asn Met Asn Asn Gly Asn Asp Lys Ser His Arg Asp Val
 115 120 125
 Ser Ser Glu Glu Asn Arg Tyr Phe Ser Val Glu Lys Asn Glu Tyr Pro
 130 135 140
 Thr Lys Leu Asn Gly Lys Ala Val Thr Thr Glu Asp Gln Thr Gln Lys
 145 150 155 160
 Arg Arg Glu Asp Tyr Tyr Met Pro Arg Leu Asp Lys Phe Val Thr Glu
 165 170 175
 Val Ala Pro Ile Glu Ala Ser Thr Ala Ser Ser Asp Ala Gly Thr Tyr
 180 185 190
 Asn Asp Gln Asn Lys Tyr Pro Ala Phe Val Arg Leu Gly Ser Gly Thr
 195 200 205
 Gln Phe Ile Tyr Lys Lys Gly Asp Asn Tyr Ser Leu Ile Leu Asn Asn
 210 215 220
 His Glu Val Gly Gly Asn Asn Leu Lys Leu Val Gly Asp Ala Tyr Thr
 225 230 235 240
 Tyr Gly Ile Ala Gly Thr Pro Tyr Lys Val Asn His Glu Asn Asn Gly
 245 250 255
 Leu Ile Gly Phe Gly Asn Ser Lys Glu Glu His Ser Asp Pro Lys Gly
 260 265 270
 Ile Leu Ser Gln Asp Pro Leu Thr Asn Tyr Ala Val Leu Gly Asp Ser
 275 280 285
 Gly Ser Pro Leu Phe Val Tyr Asp Arg Glu Lys Gly Lys Trp Leu Phe
 290 295 300
 Leu Gly Ser Tyr Asp Phe Trp Ala Gly Tyr Asn Lys Lys Ser Trp Gln
 305 310 315 320
 Glu Trp Asn Ile Tyr Lys Pro Glu Phe Ala Lys Thr Val Leu Asp Lys
 325 330 335
 Asp Thr Ala Gly Ser Leu Ile Gly Ser Asn Thr Gln Tyr Asn Trp Asn
 340 345 350
 Pro Thr Gly Lys Thr Ser Val Ile Ser Asn Gly Ser Glu Ser Leu Asn
 355 360 365
 Val Asp Leu Phe Asp Ser Ser Gln Asp Thr Asp Ser Lys Lys Asn Asn
 370 375 380
 His Gly Lys Ser Val Thr Leu Arg Gly Ser Gly Thr Leu Thr Leu Asn
 385 390 395 400
 Asn Asn Ile Asp Gln Gly Ala Gly Gly Leu Phe Phe Glu Gly Asp Tyr
 405 410 415
 Glu Val Lys Gly Thr Ser Asp Ser Thr Thr Trp Lys Gly Ala Gly Val
 420 425 430
 Ser Val Ala Asp Gly Lys Thr Val Thr Trp Lys Val His Asn Pro Lys
 435 440 445
 Ser Asp Arg Leu Ala Lys Ile Gly Lys Gly Thr Leu Ile Val Glu Gly
 450 455 460
 Lys Gly Glu Asn Lys Gly Ser Leu Lys Val Gly Asp Gly Thr Val Ile
 465 470 475 480
 Leu Lys Gln Gln Ala Asp Ala Asn Asn Lys Val Lys Ala Phe Ser Gln
 485 490 495

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Val	Gly	Ile	Val	Ser	Gly	Arg	Ser	Thr	Val	Val	Leu	Asn	Asp	Asp	Lys
			500					505					510		
Gln	Val	Asp	Pro	Asn	Ser	Ile	Tyr	Phe	Gly	Phe	Arg	Gly	Gly	Arg	Leu
		515					520					525			
Asp	Ala	Asn	Gly	Asn	Asn	Leu	Thr	Phe	Glu	His	Ile	Arg	Asn	Ile	Asp
	530					535					540				
Asp	Gly	Ala	Arg	Leu	Val	Asn	His	Asn	Thr	Ser	Lys	Thr	Ser	Thr	Val
545					550					555					560
Thr	Ile	Thr	Gly	Glu	Ser	Leu	Ile	Thr	Asp	Pro	Asn	Thr	Ile	Thr	Pro
				565					570					575	
Tyr	Asn	Ile	Asp	Ala	Pro	Asp	Glu	Asp	Asn	Pro	Tyr	Ala	Phe	Arg	Arg
			580					585					590		
Ile	Lys	Asp	Gly	Gly	Gln	Leu	Tyr	Leu	Asn	Leu	Glu	Asn	Tyr	Thr	Tyr
		595					600					605			
Tyr	Ala	Leu	Arg	Lys	Gly	Ala	Ser	Thr	Arg	Ser	Glu	Leu	Pro	Lys	Asn
	610					615					620				
Ser	Gly	Glu	Ser	Asn	Glu	Asn	Trp	Leu	Tyr	Met	Gly	Lys	Thr	Ser	Asp
625					630					635					640
Ala	Ala	Lys	Arg	Asn	Val	Met	Asn	His	Ile	Asn	Asn	Glu	Arg	Met	Asn
				645					650					655	
Gly	Phe	Asn	Gly	Tyr	Phe	Gly	Glu	Glu	Glu	Gly	Lys	Asn	Asn	Gly	Asn
			660					665					670		
Leu	Asn	Val	Thr	Phe	Lys	Gly	Lys	Ser	Glu	Gln	Asn	Arg	Phe	Leu	Leu
		675					680					685			
Thr	Gly	Gly	Thr	Asn	Leu	Asn	Gly	Asp	Leu	Lys	Val	Glu	Lys	Gly	Thr
	690					695					700				
Leu	Phe	Leu	Ser	Gly	Arg	Pro	Thr	Pro	His	Ala	Arg	Asp	Ile	Ala	Gly
705					710					715					720
Ile	Ser	Ser	Thr	Lys	Lys	Asp	Gln	His	Phe	Ala	Glu	Asn	Asn	Glu	Val
				725					730					735	
Val	Val	Glu	Asp	Asp	Trp	Ile	Asn	Arg	Asn	Phe	Lys	Ala	Thr	Asn	Ile
			740					745						750	
Asn	Val	Thr	Asn	Asn	Ala	Thr	Leu	Tyr	Ser	Gly	Arg	Asn	Val	Ala	Asn
		755					760					765			
Ile	Thr	Ser	Asn	Ile	Thr	Ala	Ser	Asp	Asn	Ala	Lys	Val	His	Ile	Gly
	770					775					780				
Tyr	Lys	Ala	Gly	Asp	Thr	Val	Cys	Val	Arg	Ser	Asp	Tyr	Thr	Gly	Tyr
785					790					795					800
Val	Thr	Cys	Thr	Thr	Asp	Lys	Leu	Ser	Asp	Lys	Ala	Leu	Asn	Ser	Phe
				805					810					815	
Asn	Ala	Thr	Asn	Val	Ser	Gly	Asn	Val	Asn	Leu	Ser	Gly	Asn	Ala	Asn
			820					825					830		
Phe	Val	Leu	Gly	Lys	Ala	Asn	Leu	Phe	Gly	Thr	Ile	Ser	Gly	Thr	Gly
		835					840					845			
Asn	Ser	Gln	Val	Arg	Leu	Thr	Glu	Asn	Ser	His	Trp	His	Leu	Thr	Gly
						855					860				
Asp	Ser	Asn	Val	Asn	Gln	Leu	Asn	Leu	Asp	Lys	Gly	His	Ile	His	Leu
865					870					875					880
Asn	Ala	Gln	Asn	Asp	Ala	Asn	Lys	Val	Thr	Thr	Tyr	Asn	Thr	Leu	Thr
				885					890					895	
Val	Asn	Ser	Leu	Ser	Gly	Asn	Gly	Ser	Phe	Tyr	Tyr	Leu	Thr	Asp	Leu
			900					905						910	
Ser	Asn	Lys	Gln	Gly	Asp	Lys	Val	Val	Val	Thr	Lys	Ser	Ala	Thr	Gly

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915			920			925									
Asn	Phe	Thr	Leu	Gln	Val	Ala	Asp	Lys	Thr	Gly	Glu	Pro	Thr	Lys	Asn
	930						935				940				
Glu	Leu	Thr	Leu	Phe	Asp	Ala	Ser	Asn	Ala	Thr	Arg	Asn	Asn	Leu	Asn
	945				950					955					960
Val	Ser	Leu	Val	Gly	Asn	Thr	Val	Asp	Leu	Gly	Ala	Trp	Lys	Tyr	Lys
				965						970					975
Leu	Arg	Asn	Val	Asn	Gly	Arg	Tyr	Asp	Leu	Tyr	Asn	Pro	Glu	Val	Glu
			980					985					990		
Lys	Arg	Asn	Gln	Thr	Val	Asp	Thr	Thr	Asn	Ile	Thr	Thr	Pro	Asn	Asn
		995						1000					1005		
Ile	Gln	Ala	Asp	Val	Pro	Ser	Val	Pro	Ser	Asn	Asn	Glu	Glu	Ile	Ala
	1010						1015				1020				
Arg	Val	Glu	Thr	Pro	Val	Pro	Pro	Pro	Ala	Pro	Ala	Thr	Pro	Ser	Glu
	1025				1030					1035					1040
Thr	Thr	Glu	Thr	Val	Ala	Glu	Asn	Ser	Lys	Gln	Glu	Ser	Lys	Thr	Val
				1045						1050					1055
Glu	Lys	Asn	Glu	Gln	Asp	Ala	Thr	Glu	Thr	Thr	Ala	Gln	Asn	Gly	Glu
			1060					1065						1070	
Val	Ala	Glu	Glu	Ala	Lys	Pro	Ser	Val	Lys	Ala	Asn	Thr	Gln	Thr	Asn
		1075						1080					1085		
Glu	Val	Ala	Gln	Ser	Gly	Ser	Glu	Thr	Glu	Glu	Thr	Gln	Thr	Thr	Glu
	1090						1095				1100				
Ile	Lys	Glu	Thr	Ala	Lys	Val	Glu	Lys	Glu	Glu	Lys	Ala	Lys	Val	Glu
	1105				1110					1115					1120
Lys	Glu	Glu	Lys	Ala	Lys	Val	Glu	Lys	Asp	Glu	Ile	Gln	Glu	Ala	Pro
				1125						1130				1135	
Gln	Met	Ala	Ser	Glu	Thr	Ser	Pro	Lys	Gln	Ala	Lys	Pro	Ala	Pro	Lys
		1140						1145					1150		
Glu	Val	Ser	Thr	Asp	Thr	Lys	Val	Glu	Glu	Thr	Gln	Val	Gln	Ala	Gln
		1155					1160						1165		
Pro	Gln	Thr	Gln	Ser	Thr	Thr	Val	Ala	Ala	Ala	Glu	Ala	Thr	Ser	Pro
	1170						1175				1180				
Asn	Ser	Lys	Pro	Ala	Glu	Glu	Thr	Gln	Pro	Ser	Glu	Lys	Thr	Asn	Ala
	1185				1190					1195					1200
Glu	Pro	Val	Thr	Pro	Val	Val	Ser	Lys	Asn	Gln	Thr	Glu	Asn	Thr	Thr
				1205						1210					1215
Asp	Gln	Pro	Thr	Glu	Arg	Glu	Lys	Thr	Ala	Lys	Val	Glu	Thr	Glu	Lys
			1220					1225					1230		
Thr	Gln	Glu	Pro	Pro	Gln	Val	Ala	Ser	Gln	Ala	Ser	Pro	Lys	Gln	Glu
		1235					1240						1245		
Gln	Ser	Glu	Thr	Val	Gln	Pro	Gln	Ala	Val	Leu	Glu	Ser	Glu	Asn	Val
		1250					1255				1260				
Pro	Thr	Val	Asn	Asn	Ala	Glu	Glu	Val	Gln	Ala	Gln	Leu	Gln	Thr	Gln
	1265				1270					1275					1280
Thr	Ser	Ala	Thr	Val	Ser	Thr	Lys	Gln	Pro	Ala	Pro	Glu	Asn	Ser	Ile
			1285							1290				1295	
Asn	Thr	Gly	Ser	Ala	Thr	Ala	Ile	Thr	Glu	Thr	Ala	Glu	Lys	Ser	Asp
		1300						1305					1310		
Lys	Pro	Gln	Thr	Glu	Thr	Ala	Ala	Ser	Thr	Glu	Asp	Ala	Ser	Gln	His
		1315						1320					1325		
Lys	Ala	Asn	Thr	Val	Ala	Asp	Asn	Ser	Val	Ala	Asn	Asn	Ser	Glu	Ser
	1330						1335				1340				

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Ser Glu Pro Lys Ser Arg Arg Arg Arg Ser Ile Ser Gln Pro Gln Glu
 1345 1350 1355 1360
 Thr Ser Ala Glu Glu Thr Thr Ala Ala Ser Thr Asp Glu Thr Thr Ile
 1365 1370 1375
 Ala Asp Asn Ser Lys Arg Ser Lys Pro Asn Arg Arg Ser Arg Arg Ser
 1380 1385 1390
 Val Arg Ser Glu Pro Thr Val Thr Asn Gly Ser Asp Arg Ser Thr Val
 1395 1400 1405
 Ala Leu Arg Asp Leu Thr Ser Thr Asn Thr Asn Ala Val Ile Ser Asp
 1410 1415 1420
 Ala Met Ala Lys Ala Gln Phe Val Ala Leu Asn Val Gly Lys Ala Val
 1425 1430 1435 1440
 Ser Gln His Ile Ser Gln Leu Glu Met Asn Asn Glu Gly Gln Tyr Asn
 1445 1450 1455
 Val Trp Val Ser Asn Thr Ser Met Asn Glu Asn Tyr Ser Ser Ser Gln
 1460 1465 1470
 Tyr Arg Arg Phe Ser Ser Lys Ser Thr Gln Thr Gln Leu Gly Trp Asp
 1475 1480 1485
 Gln Thr Ile Ser Asn Asn Val Gln Leu Gly Gly Val Phe Thr Tyr Val
 1490 1495 1500
 Arg Asn Ser Asn Asn Phe Asp Lys Ala Ser Ser Lys Asn Thr Leu Ala
 1505 1510 1515 1520
 Gln Val Asn Phe Tyr Ser Lys Tyr Tyr Ala Asp Asn His Trp Tyr Leu
 1525 1530 1535
 Gly Ile Asp Leu Gly Tyr Gly Lys Phe Gln Ser Asn Leu Lys Thr Asn
 1540 1545 1550
 His Asn Ala Lys Phe Ala Arg His Thr Ala Gln Phe Gly Leu Thr Ala
 1555 1560 1565
 Gly Lys Ala Phe Asn Leu Gly Asn Phe Gly Ile Thr Pro Ile Val Gly
 1570 1575 1580
 Val Arg Tyr Ser Tyr Leu Ser Asn Ala Asn Phe Ala Leu Ala Lys Asp
 1585 1590 1595 1600
 Arg Ile Lys Val Asn Pro Ile Ser Val Lys Thr Ala Phe Ala Gln Val
 1605 1610 1615
 Asp Leu Ser Tyr Thr Tyr His Leu Gly Glu Phe Ser Val Thr Pro Ile
 1620 1625 1630
 Leu Ser Ala Arg Tyr Asp Thr Asn Gln Gly Ser Gly Lys Ile Asn Val
 1635 1640 1645
 Asn Gln Tyr Asp Phe Ala Tyr Asn Val Glu Asn Gln Gln Gln Tyr Asn
 1650 1655 1660
 Ala Gly Leu Lys Leu Lys Tyr His Asn Val Lys Leu Ser Leu Ile Gly
 1665 1670 1675 1680
 Gly Leu Thr Lys Ala Lys Gln Ala Glu Lys Gln Lys Thr Ala Glu Leu
 1685 1690 1695
 Lys Leu Ser Phe Ser Phe
 1700

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1848 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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Met Leu Asn Lys Lys Phe Lys Leu Asn Phe Ile Ala Leu Thr Val Ala
 1 5 10 15
 Tyr Ala Leu Thr Pro Tyr Thr Glu Ala Ala Leu Val Arg Asp Asp Val
 20 25 30
 Asp Tyr Gln Ile Phe Arg Asp Phe Ala Glu Asn Lys Gly Lys Phe Ser
 35 40 45
 Val Gly Ala Thr Asn Val Glu Val Arg Asp Lys Lys Asn Gln Ser Leu
 50 55 60
 Gly Ser Ala Leu Pro Asn Gly Ile Pro Met Ile Asp Phe Ser Val Val
 65 70 75 80
 Asp Val Asp Lys Arg Ile Ala Thr Leu Val Asn Pro Gln Tyr Val Val
 85 90 95
 Gly Val Lys His Val Ser Asn Gly Val Ser Glu Leu His Phe Gly Asn
 100 105 110
 Leu Asn Gly Asn Met Asn Asn Gly Asn Ala Lys Ser His Arg Asp Val
 115 120 125
 Ser Ser Glu Glu Asn Arg Tyr Tyr Thr Val Glu Lys Asn Asn Phe Pro
 130 135 140
 Thr Glu Asn Val Thr Ser Phe Thr Lys Glu Glu Gln Asp Ala Gln Lys
 145 150 155 160
 Arg Arg Glu Asp Tyr Tyr Met Pro Arg Leu Asp Lys Phe Val Thr Glu
 165 170 175
 Val Ala Pro Ile Glu Ala Ser Thr Ala Asn Asn Asn Lys Gly Glu Tyr
 180 185 190
 Asn Asn Ser Asp Lys Tyr Pro Ala Phe Val Arg Leu Gly Ser Gly Thr
 195 200 205
 Gln Phe Ile Tyr Lys Lys Gly Ser Arg Tyr Gln Leu Ile Leu Thr Glu
 210 215 220
 Lys Asp Lys Gln Gly Asn Leu Leu Arg Asn Trp Asp Val Gly Gly Asp
 225 230 235 240
 Asn Leu Glu Leu Val Gly Asn Ala Tyr Thr Tyr Gly Ile Ala Gly Thr
 245 250 255
 Pro Tyr Lys Val Asn His Glu Asn Asn Gly Leu Ile Gly Phe Gly Asn
 260 265 270
 Ser Lys Glu Glu His Ser Asp Pro Lys Gly Ile Leu Ser Gln Asp Pro
 275 280 285
 Leu Thr Asn Tyr Ala Val Leu Gly Asp Ser Gly Ser Pro Leu Phe Val
 290 295 300
 Tyr Asp Arg Glu Lys Gly Lys Trp Leu Phe Leu Gly Ser Tyr Asp Phe
 305 310 315 320
 Trp Ala Gly Tyr Asn Lys Lys Ser Trp Gln Glu Trp Asn Ile Tyr Lys
 325 330 335
 His Glu Phe Ala Glu Lys Ile Tyr Gln Gln Tyr Ser Ala Gly Ser Leu
 340 345 350
 Ile Gly Ser Asn Thr Gln Tyr Thr Trp Gln Ala Thr Gly Ser Thr Ser
 355 360 365
 Thr Ile Thr Gly Gly Gly Glu Pro Leu Ser Val Asp Leu Thr Asp Gly
 370 375 380
 Lys Asp Lys Pro Asn His Gly Lys Ser Ile Thr Leu Lys Gly Ser Gly
 385 390 395 400
 Thr Leu Thr Leu Asn Asn His Ile Asp Gln Gly Ala Gly Gly Leu Phe
 405 410 415

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Phe	Glu	Gly	Asp	Tyr	Glu	Val	Lys	Gly	Thr	Ser	Asp	Ser	Thr	Thr	Trp
			420					425					430		
Lys	Gly	Ala	Gly	Val	Ser	Val	Ala	Asp	Gly	Lys	Thr	Val	Thr	Trp	Lys
		435					440					445			
Val	His	Asn	Pro	Lys	Tyr	Asp	Arg	Leu	Ala	Lys	Ile	Gly	Lys	Gly	Thr
	450					455					460				
Leu	Val	Val	Glu	Gly	Lys	Gly	Lys	Asn	Glu	Gly	Leu	Leu	Lys	Val	Gly
465					470					475					480
Asp	Gly	Thr	Val	Ile	Leu	Lys	Gln	Lys	Ala	Asp	Ala	Asn	Asn	Lys	Val
				485					490					495	
Gln	Ala	Phe	Ser	Gln	Val	Gly	Ile	Val	Ser	Gly	Arg	Ser	Thr	Leu	Val
			500					505					510		
Leu	Asn	Asp	Asp	Lys	Gln	Val	Asp	Pro	Asn	Ser	Ile	Tyr	Phe	Gly	Phe
		515					520					525			
Arg	Gly	Gly	Arg	Leu	Asp	Leu	Asn	Gly	Asn	Ser	Leu	Thr	Phe	Asp	His
	530					535					540				
Ile	Arg	Asn	Ile	Asp	Asp	Gly	Ala	Arg	Val	Val	Asn	His	Asn	Met	Thr
545					550					555					560
Asn	Thr	Ser	Asn	Ile	Thr	Ile	Thr	Gly	Glu	Ser	Leu	Ile	Thr	Asn	Pro
				565					570					575	
Asn	Thr	Ile	Thr	Ser	Tyr	Asn	Ile	Glu	Ala	Gln	Asp	Asp	Asp	His	Pro
			580					585					590		
Leu	Arg	Ile	Arg	Ser	Ile	Pro	Tyr	Arg	Gln	Leu	Tyr	Phe	Asn	Gln	Asp
		595					600					605			
Asn	Arg	Ser	Tyr	Tyr	Thr	Leu	Lys	Lys	Gly	Ala	Ser	Thr	Arg	Ser	Glu
	610					615					620				
Leu	Pro	Gln	Asn	Ser	Gly	Glu	Ser	Asn	Glu	Asn	Trp	Leu	Tyr	Met	Gly
625					630					635					640
Arg	Thr	Ser	Asp	Ala	Ala	Lys	Arg	Asn	Val	Met	Asn	His	Ile	Asn	Asn
				645					650					655	
Glu	Arg	Met	Asn	Gly	Phe	Asn	Gly	Tyr	Phe	Gly	Glu	Glu	Glu	Thr	Lys
			660					665					670		
Ala	Thr	Gln	Asn	Gly	Lys	Leu	Asn	Val	Thr	Phe	Asn	Gly	Lys	Ser	Asp
		675					680					685			
Gln	Asn	Arg	Phe	Leu	Leu	Thr	Gly	Gly	Thr	Asn	Leu	Asn	Gly	Asp	Leu
690						695					700				
Asn	Val	Glu	Lys	Gly	Thr	Leu	Phe	Leu	Ser	Gly	Arg	Pro	Thr	Pro	His
705					710					715					720
Ala	Arg	Asp	Ile	Ala	Gly	Ile	Ser	Ser	Thr	Lys	Lys	Asp	Pro	His	Phe
				725					730					735	
Thr	Glu	Asn	Asn	Glu	Val	Val	Val	Glu	Asp	Asp	Trp	Ile	Asn	Arg	Asn
				740				745					750		
Phe	Lys	Ala	Thr	Thr	Met	Asn	Val	Thr	Gly	Asn	Ala	Ser	Leu	Tyr	Ser
		755					760					765			
Gly	Arg	Asn	Val	Ala	Asn	Ile	Thr	Ser	Asn	Ile	Thr	Ala	Ser	Asn	Asn
	770					775					780				
Ala	Gln	Val	His	Ile	Gly	Tyr	Lys	Thr	Gly	Asp	Thr	Val	Cys	Val	Arg
785					790					795					800
Ser	Asp	Tyr	Thr	Gly	Tyr	Val	Thr	Cys	His	Asn	Ser	Asn	Leu	Ser	Glu
				805					810				815		
Lys	Ala	Leu	Asn	Ser	Phe	Asn	Pro	Thr	Asn	Leu	Arg	Gly	Asn	Val	Asn
			820					825					830		
Leu	Thr	Glu	Asn	Ala	Ser	Phe	Thr	Leu	Gly	Lys	Ala	Asn	Leu	Phe	Gly

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835					840					845					
Thr	Ile	Gln	Ser	Ile	Gly	Thr	Ser	Gln	Val	Asn	Leu	Lys	Glu	Asn	Ser
850						855					860				
His	Trp	His	Leu	Thr	Gly	Asn	Ser	Asn	Val	Asn	Gln	Leu	Asn	Leu	Thr
865					870					875					880
Asn	Gly	His	Ile	His	Leu	Asn	Ala	Gln	Asn	Asp	Ala	Asn	Lys	Val	Thr
				885					890					895	
Thr	Tyr	Asn	Thr	Leu	Thr	Val	Asn	Ser	Leu	Ser	Gly	Asn	Gly	Ser	Phe
			900					905					910		
Tyr	Tyr	Trp	Val	Asp	Phe	Thr	Asn	Asn	Lys	Ser	Asn	Lys	Val	Val	Val
		915					920					925			
Asn	Lys	Ser	Ala	Thr	Gly	Asn	Phe	Thr	Leu	Gln	Val	Ala	Asp	Lys	Thr
930						935					940				
Gly	Glu	Pro	Asn	His	Asn	Glu	Leu	Thr	Leu	Phe	Asp	Ala	Ser	Asn	Ala
945					950					955					960
Thr	Arg	Asn	Asn	Leu	Glu	Val	Thr	Leu	Ala	Asn	Gly	Ser	Val	Asp	Arg
				965					970					975	
Gly	Ala	Trp	Lys	Tyr	Lys	Leu	Arg	Asn	Val	Asn	Gly	Arg	Tyr	Asp	Leu
			980					985					990		
Tyr	Asn	Pro	Glu	Val	Glu	Lys	Arg	Asn	Gln	Thr	Val	Asp	Thr	Thr	Asn
		995						1000					1005		
Ile	Thr	Thr	Pro	Asn	Asp	Ile	Gln	Ala	Asp	Ala	Pro	Ser	Ala	Gln	Ser
1010						1015					1020				
Asn	Asn	Glu	Glu	Ile	Ala	Arg	Val	Glu	Thr	Pro	Val	Pro	Pro	Pro	Ala
1025					1030					1035					1040
Pro	Ala	Thr	Glu	Ser	Ala	Ile	Ala	Ser	Glu	Gln	Pro	Glu	Thr	Arg	Pro
				1045					1050					1055	
Ala	Glu	Thr	Ala	Gln	Pro	Ala	Met	Glu	Glu	Thr	Asn	Thr	Ala	Asn	Ser
			1060					1065					1070		
Thr	Glu	Thr	Ala	Pro	Lys	Ser	Asp	Thr	Ala	Thr	Gln	Thr	Glu	Asn	Pro
			1075				1080						1085		
Asn	Ser	Glu	Ser	Val	Pro	Ser	Glu	Thr	Thr	Glu	Lys	Val	Ala	Glu	Asn
1090						1095					1100				
Pro	Pro	Gln	Glu	Asn	Glu	Thr	Val	Ala	Lys	Asn	Glu	Gln	Glu	Ala	Thr
1105					1110					1115					1120
Glu	Pro	Thr	Pro	Gln	Asn	Gly	Glu	Val	Ala	Lys	Glu	Asp	Gln	Pro	Thr
				1125					1130					1135	
Val	Glu	Ala	Asn	Thr	Gln	Thr	Asn	Glu	Ala	Thr	Gln	Ser	Glu	Gly	Lys
			1140					1145					1150		
Thr	Glu	Glu	Thr	Gln	Thr	Ala	Glu	Thr	Lys	Ser	Glu	Pro	Thr	Glu	Ser
			1155				1160						1165		
Val	Thr	Val	Ser	Glu	Asn	Gln	Pro	Glu	Lys	Thr	Val	Ser	Gln	Ser	Thr
1170						1175					1180				
Glu	Asp	Lys	Val	Val	Val	Glu	Lys	Glu	Glu	Lys	Ala	Lys	Val	Glu	Thr
1185					1190					1195					1200
Glu	Glu	Thr	Gln	Lys	Ala	Pro	Gln	Val	Thr	Ser	Lys	Glu	Pro	Pro	Lys
				1205					1210					1215	
Gln	Ala	Glu	Pro	Ala	Pro	Glu	Glu	Val	Pro	Thr	Asp	Thr	Asn	Ala	Glu
			1220					1225					1230		
Glu	Ala	Gln	Ala	Leu	Gln	Gln	Thr	Gln	Pro	Thr	Thr	Val	Ala	Ala	Ala
			1235				1240						1245		
Glu	Thr	Thr	Ser	Pro	Asn	Ser	Lys	Pro	Ala	Glu	Glu	Thr	Gln	Gln	Pro
1250						1255						1260			

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Ser Glu Lys Thr Asn Ala Glu Pro Val Thr Pro Val Val Ser Glu Asn
 1265 1270 1275 1280

Thr Ala Thr Gln Pro Thr Glu Thr Glu Glu Thr Ala Lys Val Glu Lys
 1285 1290 1295

Glu Lys Thr Gln Glu Val Pro Gln Val Ala Ser Gln Glu Ser Pro Lys
 1300 1305 1310

Gln Glu Gln Pro Ala Ala Lys Pro Gln Ala Gln Thr Lys Pro Gln Ala
 1315 1320 1325

Glu Pro Ala Arg Glu Asn Val Leu Thr Thr Lys Asn Val Gly Glu Pro
 1330 1335 1340

Gln Pro Gln Ala Gln Pro Gln Thr Gln Ser Thr Ala Val Pro Thr Thr
 1345 1350 1355 1360

Gly Glu Thr Ala Ala Asn Ser Lys Pro Ala Ala Lys Pro Gln Ala Gln
 1365 1370 1375

Ala Lys Pro Gln Thr Glu Pro Ala Arg Glu Asn Val Ser Thr Val Asn
 1380 1385 1390

Thr Lys Glu Pro Gln Ser Gln Thr Ser Ala Thr Val Ser Thr Glu Gln
 1395 1400 1405

Pro Ala Lys Glu Thr Ser Ser Asn Val Glu Gln Pro Ala Pro Glu Asn
 1410 1415 1420

Ser Ile Asn Thr Gly Ser Ala Thr Thr Met Thr Glu Thr Ala Glu Lys
 1425 1430 1435 1440

Ser Asp Lys Pro Gln Met Glu Thr Val Thr Glu Asn Asp Arg Gln Pro
 1445 1450 1455

Glu Ala Asn Thr Val Ala Asp Asn Ser Val Ala Asn Asn Ser Glu Ser
 1460 1465 1470

Ser Glu Ser Lys Ser Arg Arg Arg Arg Ser Val Ser Gln Pro Lys Glu
 1475 1480 1485

Thr Ser Ala Glu Glu Thr Thr Val Ala Ser Thr Gln Glu Thr Thr Val
 1490 1495 1500

Asp Asn Ser Val Ser Thr Pro Lys Pro Arg Ser Arg Arg Thr Arg Arg
 1505 1510 1515 1520

Ser Val Gln Thr Asn Ser Tyr Glu Pro Val Glu Leu Pro Thr Glu Asn
 1525 1530 1535

Ala Glu Asn Ala Glu Asn Val Gln Ser Gly Asn Asn Val Ala Asn Ser
 1540 1545 1550

Gln Pro Ala Leu Arg Asn Leu Thr Ser Lys Asn Thr Asn Ala Val Ile
 1555 1560 1565

Ser Asn Ala Met Ala Lys Ala Gln Phe Val Ala Leu Asn Val Gly Lys
 1570 1575 1580

Ala Val Ser Gln His Ile Ser Gln Leu Glu Met Asn Asn Glu Gly Gln
 1585 1590 1595 1600

Tyr Asn Val Trp Ile Ser Asn Thr Ser Met Asn Lys Asn Tyr Ser Ser
 1605 1610 1615

Glu Gln Tyr Arg Arg Phe Ser Ser Lys Ser Thr Gln Thr Gln Leu Gly
 1620 1625 1630

Trp Asp Gln Thr Ile Ser Asn Asn Val Gln Leu Gly Gly Val Phe Thr
 1635 1640 1645

Tyr Val Arg Asn Ser Asn Asn Phe Asp Lys Ala Ser Ser Lys Asn Thr
 1650 1655 1660

Leu Ala Gln Val Asn Phe Tyr Ser Lys Tyr Tyr Ala Asp Asn His Trp
 1665 1670 1675 1680

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Tyr Leu Gly Ile Asp Leu Gly Tyr Gly Lys Phe Gln Ser Asn Leu Gln
1685 1690 1695

Thr Asn Asn Asn Ala Lys Phe Ala Arg His Thr Ala Gln Ile Gly Leu
1700 1705 1710

Thr Ala Gly Lys Ala Phe Asn Leu Gly Asn Phe Ala Val Lys Pro Thr
1715 1720 1725

Val Gly Val Arg Tyr Ser Tyr Leu Ser Asn Ala Asp Phe Ala Leu Ala
1730 1735 1740

Gln Asp Arg Ile Lys Val Asn Pro Ile Ser Val Lys Thr Ala Phe Ala
1745 1750 1755 1760

Gln Val Asp Leu Ser Tyr Thr Tyr His Leu Gly Glu Phe Ser Ile Thr
1765 1770 1775

Pro Ile Leu Ser Ala Arg Tyr Asp Ala Asn Gln Gly Asn Gly Lys Ile
1780 1785 1790

Asn Val Ser Val Tyr Asp Phe Ala Tyr Asn Val Glu Asn Gln Gln Gln
1795 1800 1805

Tyr Asn Ala Gly Leu Lys Leu Lys Tyr His Asn Val Lys Leu Ser Leu
1810 1815 1820

Ile Gly Gly Leu Thr Lys Ala Lys Gln Ala Glu Lys Gln Lys Thr Ala
1825 1830 1835 1840

Glu Val Lys Leu Ser Phe Ser Phe
1845

(2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Gly Asp Ser Gly Ser Pro Met Phe
1 5

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Gly Asp Ser Gly Ser Pro Leu Phe
1 5

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

His Thr Tyr Phe Gly Ile Asp
1 5

What is claimed is:

1. A method of producing a Haemophilus adhesion and penetration protein comprising:

- a) culturing a host cell transformed with an expression vector comprising a nucleic acid encoding a Haemo-

philus adhesion and penetration protein, whose non-coding nucleic acid strand will hybridize to a nucleic acid strand having a coding sequence as shown in SEQ ID NO:1 under the high stringency conditions of washes at 0.1×SSC at 65° C. for 2 hours; and

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b) expressing said nucleic acid to produce a recombinant Haemophilus adhesion and penetration protein.

2. The method of claim 1, wherein said nucleic acid has the sequence as shown in SEQ ID NO:1.

3. The method of claim 1, wherein the recombinant Haemophilus adhesion and penetration protein has an amino acid sequence as shown in SEQ ID NO:2.

4. The method of claim 1, wherein said host cell is selected from the group consisting of yeast, bacteria, archeobacteria, fungi, insect cells, and animal cells.

5. The method of claim 4, wherein said host cell is a bacteria cell.

6. The method of claim 5, wherein said bacterial cell is selected from the group consisting of *Bacillus subtilis*, *E. coli*, *Streptococcus cremoris*, and *Streptococcus lividans*.

7. The method of claim 4, wherein said host cell is a yeast cell.

8. The method of claim 7, wherein said yeast cell is selected from the group consisting of *Saccharomyces*

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cerevisiae, *Candida albicans*, *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia quillerimondii*, *Pichia pastoris*, *Schizosaccharomyces pombe*, and *Yarrowia lipolytica*.

9. The method of claim 4, wherein said host cell is an insect cell.

10. The method of claim 9, wherein said insect cell is a *Drosophila melanogaster* cell.

11. The method of claim 4, wherein said host cell is an animal cell.

12. The method of claim 11, wherein said animal cell is selected from the group consisting of HeLa cells, immortalized mammalian myeloid cells and immortalized mammalian lymphoid cells.

13. The method of claim 1, wherein said recombinant Haemophilus adhesion and penetration protein is secreted from said host cell.

* * * * *